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Polyculture affects biomass production of component species but not total standing biomass and soil carbon stocks in a temperate forest plantation

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Abstract

Key message: Overyielding of stand biomass did not occur in a tree polyculture comprised of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica* selected for contrasting traits. This was due to antagonistic interactions between the component species. Fine root dynamics and soil C stocks were unaffected by species mixture.

Context: Increasing CO_2 fixation in tree biomass through afforestation and forest management actions has potential for cost-effective climate mitigation. The influences of tree mixture on biomass production and subsequent soil C accumulation in polyculture still remain uncertain.

Aims: We studied the polyculture of *Alnus glutinosa* (L.) Gaertn, *Betula pendula* Roth and *Fagus sylvatica* L. in a plantation forest to examine the effectiveness of species mixtures as a tool for increased biomass production and soil C accumulation.

Methods: Tree biomass was estimated by developing species-specific allometric models and three years tree measurement. Fine root biomass and production were estimated by root coring and root-mesh methods. The 'Relative Yield of Mixture' approach was used to examine the mixture effect.

Results: In mixture, an additive effect was observed in *A. glutinosa* (13% increase in basal diameter relative to the monoculture), however, there was no overall effect of mixture on total standing biomass due to the suppression of *F. sylvatica* (2.75 g m^{-2} reduction in woody biomass). Fine root biomass production showed no mixture effect. The quantity and quality of soil C (top 0.5 m) was not affected by tree mixture.

Conclusions: We conclude that the contrasting growth responses of the *A. glutinosa*, *B. pendula* and *F. sylvatica* in polyculture resulted in no overyielding of standing biomass despite the complementary traits of the component species.

Introduction

Afforestation and reforestation are recommended as low-cost strategies to mitigate the anthropogenic elevation of CO_2 and the associated global impacts of climate change (Valatin and Price, 2014). Increased tree growth and biomass production due to higher tree species diversity, has been reported by some investigators, indicating positive impacts on the storage and sequestration of atmospheric C in forest

ecosystems (Bielak et al. 2014; Gamfeldt et al. 2013; Hulvey el al.2013). In plantation forestry, numerous management tools help to maximise growth and biomass production. These include species selection (Nord-Larsen and Meilby 2016; Aravanopoulos, 2010), tree composition and diversity (Belote et al. 2011; Pretzsch et al. 2010), plantation design (Kunert and Cardenas 2015; Goelz, 2001), stand density (Vanclay, 2006) etc. Nonetheless, our mechanistic understanding of species interactions within plant communities is poor and the degree to which species polyculture enhances biomass production and contributes to ecosystem C stocks remains unclear.

The potential advantage of species interactions in polyculture (in terms of biomass growth when compared with monoculture) involves competitive reduction and facilitation mechanisms (Forrester et al. 2005). Nonetheless, additive/antagonistic mixture effects resulting in no change/lower biomass production in polyculture vs. monoculture may occur due to lack of sufficient complementarity or through competition between species (Pretzsch et al. 2010; Kelty and Cameron 1995). In a recent study, Tobner et al. (2016) reported that 80% of the diversity issues in mixed species plantations were driven by the selection effect and the remaining 20% were influenced by complementarity effect in a young tree diversity experiments (www.treedivnet.ugent.be). Species density and the ratio of component species in polyculture can influence the interspecific interactions and ultimately affect stand development (Kelty, 2006). Similarly, a decrease in competition-reduction was reported by Pretzsch et al. (2012) when different spatial mixing patterns were used in a polyculture stand of *Picea abies* and *Fagus sylvatica*. These results indicate that biomass overyielding may not be achieved in polyculture due to inappropriate silvicultural interventions (Vanclay, 2006). Like aboveground biomass, polyculture can influence the biomass and vertical distribution of fine root due to niche complementarity (Tobner et al. 2013). For example, Laclau et al. (2013) reported that 27% higher total fine root biomass in a mixed stand of *Eucalyptus grandis* and *Acacia mangium* than in monoculture following a replacement series (50:50) in a 5-year-old mixed plantation. Contrasting results of no over-yielding in fine root biomass were observed by Jacob et al. (2014) in an 8-year-old temperate mixed forest of *Picea abies*, *Pseudotsuga menziesii*, *Fagus sylvatica*, *Quercus robur*. However, it is not clear whether the belowground responses synchronize the above ground biomass interactions, because only few studies addressed above and belowground simultaneously. Thus, a comprehensive assessment of above- and belowground tree biomass is crucial for a better understanding of the role of tree polyculture on ecosystem C stocks.

Tree species identity can enhance soil C stock through aboveground litter production and fine root turnover under the influence of soil and site characteristics. The magnitude of C inputs to soil by above ground litter-flux may depend on the quality and quantity of litter, biodegradation and soil properties (Jandl et al. 2007). Dead roots and rhizodeposition of tree root systems are likely more potential to stabilize soil C than aboveground litter due to slow decay rate and mineral interactions (Vesterdal et al. 2013). In addition, many previous studies reported that the stock and stability of soil C under these processes fluctuate over stand age (Chen et al. 2013). In general, most of the factors affecting C inputs are species specific, therefore the impacts of tree plantation on soil C storage are highly variable depending on the species selection. Similarly, the effect of species mixture on mineral soils C of new plantings is inconsistent. For example, Forrester et al. (2013) reported the positive influence of an *A. mearnsii* and *E. globulus* admixture on soil C stock in a young plantation forest. However, Wang et al. (2009) found no significant differences in soil C under mixed plantation of Chinese fir (*Cunninghamia lanceolata* (Lamb.) and broadleaved *Alnus cremastogyne* Burkill in an afforested land after 15 years. Contrasting results indicate that the link between tree plantation and soil C might be regulated by multiple spatial and temporal factors including tree functional traits.

Many earlier studies on mixture effect examined the interactions between two component species (Forrester et al. 2005; Laclau et al. 2013; Bielak et al. 2014). However, a three species interaction is more challenging because of the variability in competition and growth rates. In the present study, we used two fast-growing species with a late successional tree in the polyculture stand to study whether the interactions of these contrasting traits affect the biomass production and soil C stocks compared to monoculture conditions. Assessing forest biomass currently lacks methodological robustness. Since our study was confined to a single location, however, the traditional approach of tree harvesting was followed to estimate aboveground biomass, which is a more reliable method than others (Weiskittel et al. 2015). We measured litter fall to evaluate the link between active biomass and soil organic C stock under the various species compositions. The study was designed to investigate whether the polyculture of *Betula pendula* Roth, *Alnus glutinosa* (L.) Gaertn, and *Fagus sylvatica* L. increases the accumulation of tree biomass and affect soil C stocks. We hypothesized that i) combination of trees having different functional traits produces more woody and fine root biomass in polyculture than monoculture of each species. We predicted that the growth of each species would be increased in polyculture (species level), which would eventually increase standing biomass (stand level). ii) Establishment of forests comprised of tree polycultures positively affects the soil organic C stock.

Materials and Methods

Study sites and soil

Henfaes Research Centre, the research station of Bangor University, is located in the village of Abergwyngregyn, 12 km east of Bangor City, North Wales, UK (geographic position 53º14 N, 4º01 W and National Grid: SH 653 741 GB). The soil developed under more or less well-drained conditions (noncalcareous) and from unconsolidated parent materials, traditionally grouped in Britain as Brown earth (Clarke, 1940). This is classified as Dystric Cambisols according to the FAO system and recognized as the Rheidol series (Teklehaimanot et al. 2002). The topsoil is slightly acidic (pH 5.4), loamy in texture, brown and originated from glaciated shales, sandstone and mudstone at the upper portion and from glaci-fluvial deposits of clay, silt, sand and gravels at the lowland areas. The climate of the area is Hyperoceanic, and the seasonal temperature is varying between -3 to 10 °C in winter and 12 to 25 °C in summer; the mean annual rainfall is about 1000 mm (Campbell Automatic Weather Station, Campbell Scientific Ltd, Shepshed, UK).

Afforestation

The BangorDiverse site was established on 2.36 hectares of former agricultural land in March 2004, with a range of broadleaved tree species. The site consists of two adjacent blocks (Figure 1) that were previously used for growing oil seed rape and as a tree nursery. Previous to that the area was grassland. The plantation fields were established in four replicated plots 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. In the present study, we used *A. glutinosa*, *B. pendula* and *F. sylvatica*, which were selected due to their contrasting shade tolerance, successional chronology and to represent a range of taxonomic, physiological and ecological types. Two replicated plots of each species were sited in each block (hence four plots per species) and the minimum distance between any two plots of the same composition was 35 m. The site was planted with 60 cm saplings of each species with an inter-tree spacing of 1 m (10,000 trees ha^{-1}). A replacement series (with inter-tree spacing constant between treatments) with systematic hexagonal planting design (Aguiar et al. 2001) was used to maximize the mixture effect in the four polyculture plots, where each tree was surrounded by six equidistant neighbor trees (two conspecific individuals, one and three individuals of the other two species) (Figure 1). In the current study, we used 16 plots of *A. glutinosa*, *B. pendula* and *F. sylvatica* monoculture and the polyculture of these three species with equal proportion of component tree species. Hence, each species monoculture

and polyculture had four replicated plots (three single species and one mixed × 4= 16 plots; details of plantation plots are presented in Table 1). The forest floor vegetation included scattered grass and moss with a very few perennial herbs such as *Utrica dioica*, because of canopy cover of densely planted fast growing trees.

Allometric equations for biomass

Ten trees from each species (a total of 30 trees) were harvested in March 2010 to give a range of DBHs (Diameter at Breast Height, i.e. at 1.3 m above the ground level) to develop the allometric models. Two trees from each monoculture plots (2×3 species ×4 replicates) and one from mixed plots of each two blocks (1×3 species × 2 blocks) were randomly selected, given the DBH ranges of 14.5-75.4 mm, 29.9-91.5 mm, 15.0-50.2 mm for *B. pendula*, *A. glutinosa* and *F. sylvatica* respectively. Before harvesting the trees, height, DBH and basal diameter (at 22.5 cm) were measured using a telescopic measuring pole and digital callipers. As most of the trees were not perfectly round, the geometric mean of the highest and lowest diameter was calculated to estimate DBH.

After harvesting the bole, branches, dry leaves (most of the trees were leafless, hence the biomass was termed as woody biomass) and catkins were separated. The fresh weight of all separated parts was measured using an electrical balance (OHAUS, 5000 Series, Xtreme W, T51XW), the dry mass of tree components was determined from oven dried subsamples. For each species, four tree parameters (DBH, basal diameter, branch dry-mass and tree height) were considered to predict the above-ground woody biomass by developing allometric models. Based on the goodness of fit indices, basal diameter models were selected for *B. pendula* and *F. sylvatica* and the DBH model for *A. glutinosa*. The selected two parameters (basal diameter and DBH) were checked with three non-linear models, viz. power, exponential and logarithm, of which power models were found as the best fit for predicting woody biomass. The following three equations were developed for biomass estimation (Appendix Figure S1 and Table A1):

i) For *B. pendula* $Y=0.0002\ d^{2.3893}$

ii) For *A. glutinosa* $Y=0.0006\ D^{2.2775}$

iii) For *F. sylvatica* $Y= 0.0002\ d^{2.577}$

Where Y is woody biomass (kg), d is basal diameter (mm), and D is diameter at breast height (mm).

The height, DBH and basal diameter of all trees in 16 plots were measured for the years 2008, 2009 and 2010 (on average, a total of 1,805 trees were measured during March-May of each year). To estimate the

standing biomasses of each plot, the basal diameter and DBH data of individual trees were used for the above-mentioned allometric equations.

Litter collection

Litter was collected using 40 litter traps (35 cm × 35 cm plastic pot with square opening), placing two traps in each single and four traps in mixed plots. These were later mixed to one sample per plot, constituted 0.49 and 0.25 m^2 of trapping area in each mixed and monoculture plots respectively. Annual litter was collected at 15-day intervals in summer and at one-month intervals in other seasons. Collected fresh litter was sorted into leaves, branches (< 2 mm diameter), twigs and catkins oven dried.

Fine root biomass

Fine root biomass (≤ 2 mm) was sampled in April-May 2008 and 2009 before the start of the growing season using a soil corer (8 cm diameter.) from 16 mono and mixed species plots. Core samples (30 cm, split into three depth viz. 0-10, 10-20 and 20-30 cm) were collected from three randomly selected locations of each plot at an equal distance from surrounding trees. In mixed species plots, samples were collected at a point equidistant from *B. pendula*, *A. glutinosa* and *F. sylvatica* trees. Fine root biomass in each core was calculated as g m^{-2}, and the average of three cores was presented as biomass per plot. After harvesting, the samples were transported to the laboratory and stored at 4° C until washing. The whole core sample was washed through a set of mesh sieves (2.0-0.5 mm) with tap water and the fine roots were sorted following the handpicking approach based on the physical characteristics of the root matrix. Non-tree roots such as herbaceous roots were soft (non-lignified) and lighter in colour than tree roots, grass roots were white, soft and more elastic, while the moss roots were black and rigid. Some non-tree roots had fine root hairs which were absent in three tree roots in our study. To distinguish the roots of the different tree species, fine roots were compared to 'specimen roots' of three species collected during the field studies. The roots were distinguished based one colour, texture and branching pattern, often using a magnifying glass. The sorted fine roots within each soil core were dried at 70°C till constant weight and dry mass was recorded.

Root production and turnover

To estimate annual fine root (< 2 mm) production, a root-mesh technique (Godbold et al. 2003; Lukac and Godbold, 2010) was used. In this method, a nylon mesh strip (7 cm × 25 cm, 1 mm mesh size) was pushed into the ground vertically with a steel blade and hammer. Four strips were inserted at 50 cm distance from each target trees (three trees in each monoculture and three component trees in each polyculture plots were

randomly selected for this experiment) and retrieved after two intervals (6 months each). The fine roots that crossed through the net were collected and dry mass was determined. The roots of other species (non-tree species or other tree species in mixed plot) were carefully separated following the methods described in previous section. The root biomass turnover rate was calculated as annual root production divided by the mean standing root biomass.

Mixture effect in polyculture:

To determine the species interaction in polyculture, the standing aboveground woody biomass in polyculture plots (measured) was compared with a theoretical mixture (predicted) calculated from the biomass of component species growing in the monoculture plots following Relative Yield of Mixtures approach (Wilson, 1998; Smith et al. 2013). The following equation shows the predicted biomass (B_{mix}) calculation from the biomass of component species in monoculture, based on the tree number of each species in polyculture (in this case the contribution of each species in polyculture was one third):

$$B_{mix} = (1/3 * B_{Betula\ in\ mono}) + (1/3 * B_{Alnus\ in\ mono}) + (1/3 * B_{Fagas\ in\ mono})$$

Soil sampling and measuring C and N storage:

Representative soil samples were collected in different soil layers by excavating pits (100 × 100 × 100 cm) in all plantation plots during September 2008. Sixteen pits (4 × 3 monoculture and 4×1 polyculture) were excavated at the middle of each plot, equidistant from surrounding trees (in polyculture, equal distance from *A. glutinosa*, *B. pendula* and *F. sylvatica*). Composite soil samples were collected from seven layers (0-10, 10-0, 20-30, 30-40, 40-50 cm soil depth) of each pit. The bulk density of the soil was determined by core (5.5 cm diameter) sampling method with stone correction (volume of stone was estimated by water replacement process). Soil organic C and N were determined using a CN analyser (TruSpec® CN, LECO Inc.) and the stocks were estimated using bulk density data (Soil properties were presented in Appendix Table A2).

Statistical analysis

Allometric models: Each of the three monoculture and a polyculture forests had four replicated plots (n=4). Tree sampling was stratified into three DBH classes (14-75, 29-91, and 15-50 mm for *B. pendula*, *A. glutinosa* and *F. sylvatica* respectively) and from each class ten trees were randomly selected for harvest (n =10). Species-specific allometric models were developed by linking four independent variables (basal diameter, DBH, height and branch dry mass) with aboveground woody biomass using the following non-linear power regression model (Smith et al. 2012):

$Y = a X^{b}$

Where Y = woody biomass of the tree, X = a tree variable (DBH, Basal diameter, tree height or branch dry mass), a and b are regression coefficients (log transformed version: ln Y= a + b ln X with a correction factor CF). In addition, we examined the exponential ($Y = a e^{bx}$) and the logarithmic ($Y = a + b \ln X$) models to check the relationship between woody biomass and DBH, basal diameter (data not shown). To evaluate the performance to various models, we used three 'goodness of fit' criteria viz. coefficient of determination (R^2), *F*- ratio of the regression and the root mean squared error (RMSE) of the models. Development of the models, curve fit and correlation analyses were performed by the data analytical software SigmaPlot 12 (Systat Software Inc.)

Analysis of variance (ANOVA): A series of one-way between measures ANOVAs were performed to test for differences in tree and soil measures. All data were checked for normality (Shapiro-Wilk test, because the number of observation was small) and the homogeneity of variances (Levene's test). In case the assumptions for the ANOVA were not met, data were log-transformed. The dependent variables were woody biomass, fine root biomass, root production, root turnover, soil organic C and N stocks (within measures). Independent variable or grouping variable (factor) was species type in forest stands with four attributes viz. *Betula*, *Alnus* and *Fagus*, and mixture (between measures). Tukey's HSD was used for post-hoc test of multiple comparisons of the means among four factors.

T-test: An independent sample t-test was performed to compare the actual and predicted biomass in the mixed species plots, assuming the actual and predicted biomass as two different groups of cases. Similarly species level biomass, tree height and DBH between mono and polyculture plots were examined by t-test. ANOVA and t-test were conducted with SPSS 16.0 (SPSS Inc., Chicago, IL) and the level of significance $P < 0.05$ was accepted in all cases.

Correlations: Scattered plots were used to examine the relationship between soil C stock (dependent variables) and tree components, i.e. woody biomass, litter fall and fine root biomass (independent variables) and expressed by the correlation coefficient squared (R^2) to assess how much of the variability in soil C stock was shared by those tree inputs (Field, 2009).

Results

Tree growth, allometric models and biomass

All growth parameters (height, DBH, basal diameter, basal area) confirmed a significantly lower growth of *F. sylvatica* in the polyculture stand than in monoculture (Table 1). The basal diameter of *A. glutinosa* significantly increased in the mixed stand but the increases in DBH and basal area were not statistically significant. The growth of *B. pendula* was not different between the polyculture and monocultures.

Based on the high coefficient of determination (R^2), F value and the comparatively smaller RMSE than other models, the two parameter power regression curve was the best fit for the plotted values ($y = a\ x^{b}$) (Table 2). For *B. pendula* and *F. sylvatica* the basal diameter was the best predictor of woody biomass (R^2 = 0.997, F= 2746.4 and R^2= 0.985, F=517.5 for two species, respectively). For *A. glutinosa*, DBH was the best fit (R^2=0.995, F= 1491.3).

At the stand age of 4, 5 and 6 years, the production of aboveground woody biomass in the monoculture of *B. pendula* was similar to *A. glutinosa* stands (Figure 2). The biomass of *F. sylvatica* in monoculture was significantly lower than that of the two other species in all years (Figure 2). The annual biomass increment rates were 37, 44 and 124% for *B. pendula*, *A. glutinosa* and *F. sylvatica* in year 5 and 12, 24 and 39% in year 6. Despite the highest annual rates of biomass production during those two years, *F. sylvatica* had a significantly lower standing biomass stocks than other two species.

Mixture effects on biomass in polyculture:

At the species level, *B. pendula* and *A. glutinosa* exhibited no mixture effect on biomass production and the differences between polyculture and monoculture (increased in *A. glutinosa* biomass and decreased in *B. pendula*) were not statistically significant after four, five and six years of plantation (Figure 3a). However, a significant increase (13%) in basal diameter of *A. glutinosa* attributed to biomass increasing trend in polyculture stand. On the other hand, the woody biomass of *F. sylvatica* was significantly reduced in polyculture compared with monoculture over three years of experiments (Figure 3a).Thus after six years *A. glutinosa* showed insignificant increase in biomass (2.91 kg m^{-2} higher than monoculture, p=0.06) but *F. sylvatica* demonstrated significantly lower (2.76 kg m^{-2} lower than monoculture, p= 0.013) (Figure 3a).

At stand level, the difference between measured values (calculating from polyculture stand) and the predicted values (calculating from three monoculture stands) were very marginal and statistically insignificant (Figure 3b) indicating the additive mixture effect on standing biomass.

Fine root biomass, production and turnover rate

Total standing fine root biomass measured to a depth of 30 cm ranged between 59.3 ± 21.9 and 91.0 ± 38.4 gm^{-2} (Table 3). Overall, there was a significant effect of species (p=0.045) and depth (p=0.001) but no

species × depth interaction was observed. The fine root biomass of *F. sylvatica* in the 0-10 cm layer was significantly lower than that of *A. glutinosa* (p =0.036). The greatest proportion of total fine roots (78-82 %) was distributed in the upper 0-20 cm soil layer of the polyculture. Although the fine root production in *A. glutinosa* monoculture was substantially higher than both that of *F. sylvatica* and *B. pendula*, only the variation with *F. sylvatica* was statistically significant (p = 0.047). No significant mixture effect was found (Table 3).

Soil organic C and N

The C stock in *F. sylvatica* soil was significantly lower than that in *B. pendula* at 0-10 cm (p= 0.019) and lower than that in *B. pendula* and *A. glutinosa* at 10-20 cm soil layers (p=0.004 and 0.013, respectively), but no effect of species polyculture on soil C was observed (Table 4). Soil N stock did not differ among the four plantations in any layer. Soil organic C stock exhibited stronger relationship with root biomass (R^2 =0.42, p = 0.006) than aboveground biomass and litterfall (Figure 4).

Discussion

Between the two fast-growing trees, no significant variation was observed in standing woody biomass, height and DBH, indicating similar growth of these two species during young age. These results are in accordance with Claessens et al. (2010) that the growth rate of *A. glutinosa* and *B. pendula* is very high when young, and their height growth is similar up to the age of 40 years. In contrast, the above ground woody biomass in *F. sylvatica* was half that of fast-growing *B. pendula* and *A. glutinosa*. *F. sylvatica* is a shade-tolerant and late successional species, and its low biomass accumulation might be attributed to inherent characteristics of slow juvenile growth.

Species interactions in polyculture

Species interactions in polyculture can positively affect biomass production only when component species have contrasting growth characteristics and thus competitive reduction occurs (Kelty, 2006). In the present study, *B. pendula* and *A. glutinosa* are recognized as pioneer, light demanding and early successional and first growing species while *F. sylvatica* is a shade tolerant and late successional species. Theoretically, these characteristics can increase growth by develop complementarity in polyculture, however, the negative interactions between two species may happen in case of interspecific competition. Species-level comparison showed that the understory *F. sylvatica* has a significantly lower biomass and tree height (52% lower) in polyculture than monoculture. This suggests that, although it is recognized as being shade tolerant and can

survive well in the understory, the growth of young *F. sylvatica* is much higher when directly exposed to sunlight (in monoculture) than growing with diffused light in the understory of a polyculture stand. Although increased *A. glutinosa* biomass in polyculture was not statistically significant, the quantity was substantial when we compared with decreased biomass of *F. sylvatica*. The increasing trend in biomass production, *A. glutinosa* showed a positive growth trend in polyculture. Therefore our predictions of growth and biomass increase at species level in polyculture were not fully supported by the tree species we used, rather *A. glutinosa* (increased diameter growth) and *F. sylvatica* (decreased growth and biomass production) exhibited a contrasting mixture effect whilst *B. pendula* remained unchanged in polyculture.

Standing biomass in polyculture

Our study demonstrated no woody biomass over-yielding in polyculture stands, which contrasts with the general expectation of higher growth in such stands compared with monoculture. The measured and predicted (weighted) biomass in our study revealed that the yield in the polyculture stand was perfectly predictable by the values from the three monoculture stands, indicating a purely additive mixture effect on stand level biomass. However, our species level analysis revealed that this was not because of similar performances of species in mono and polycultures, but due to an opposite responses of *A. glutinosa* and *F. sylvatica* when grown in polyculture systems. This affected the overall stand level yield performances of these three species combination leading to an additive effect on woody biomass. Here, we provided evidence of occurring of contrasting interactions simultaneously in polyculture of three broadleaved species. Although tree species identities in monocultures influenced fine root dynamics, species mixture in polyculture showed no impact on stand level biomass or fine root production, presumably due to lack of sufficient root complementarity and/or young stand age. Both causes might be true in our experiment where complementarity can be insufficient due to young age of the trees (Rothe and Binkley 2001). This agrees with Domisch et al. (2015), who found no increase in fine root biomass or fine root production in a mixed stand compared to monoculture, suggesting that lack of sufficient root complementarity and young stand age could be the reasons. Our data on vertical root distribution showed that 24% of the fine root system in the polyculture was found at 20-30 cm soil depth, in compare to 19% at the same depth in monocultures. This could be an indication of niche differentiation due to contrasting rooting traits of the *B. pendula* and *A. glutinosa* in polyculture to access soil resources. The combined results of above- and belowground biomass in this study did not support our first hypothesis of increased biomass in the polyculture stands of

contrasting trees. But the trees exhibited both a synergistic and antagonistic mixture effects, indicating a complex species interaction in the polyculture systems.

Tree biomass and soil C and N stocks

A species influence on soil C stock was observed only with *F. sylvatica* soils in the top layers (0-10 and 10-20 cm). This agrees with previous studies that tree species composition affects soil organic C stock particularly in topsoil (Liu et al. 2016; Wang et al. 2009). There is an agreement that the tree species has enormous effects on the soil, mainly because of the quality and quantity of organic matter inputs through litter fall, root and soil microbial activities (Dawud et al. 2016; Vesterdal et al. 2002). Nonetheless, the impacts may be positive, negative or unpronounced depending on the characteristics of input materials, soil factors and associated C processes. For example, fresh litter inputs can contribute to organic C accumulation in soil but also enhance depletion of the soil C stock through the priming effect in plantation forests (Chen et al. 2016). Differences between SOC storage in the topsoil layers of *F. sylvatica* and the other two stands were clearly due to the low litter input and fine root production in the slow-growing *F. sylvatica* indicating effect of species identity on observed variations (Appendix Table A3). High soil organic C in mixed stand are generally attributed to high litter inputs (Forrester et al. 2013), hence no obvious variation in soil C was observed between mono and polyculture stands in the present study.

The stronger relationship between SOC stocks and fine root biomass (R^2=0.42) indicated higher control of fine roots than of aboveground biomass on the soil C stock at our forest sites. Recent experiments with ^{13}C-labelled leaf and root materials confirmed that C derived from fine roots has more potential for long-term sequestration than foliar C, presumably due to the recalcitrant nature of root compounds (Hu et al. 2016). Contrary to our second hypothesis, the influence of aboveground and fine root biomass, induced by polyculture plantations showed no significant effect on the soil C stock at 50 cm soil depth.

Limitations regarding stand age and short term experiments

We quantified mixing effects by comparing mono- and mixed species stands on the same site conditions, however, the young age of trees and the duration of experiment might limit the implication of the observed changes. The mixing effect may not remain constant during the stand development and the growth ratios of different species may vary over time (Drossler et al. 2015). For example, at the young stage, the fast growing and light demanding species capture light more efficiently but after canopy closure, the competition for light increases in polyculture stand, which may affect the growth of component species.

Similarly mixing effects on soil organic C stock may change with stand development (Liu et al. 2017). Therefore the effects of species polyculture on tree biomass and soil C at the young stage cannot be generalized over the entire life succession of the species, and rather expressed the effects based on the point of evaluation. Clearly as emphasised by other authors there is a need for longer term experiments (Bielak et al. 2014; Lu et al. 2018; Pretzsch et al. 2019).

Conclusion

Although tree species with complementary traits were selected, we did not find higher growth or biomass production in polyculture stands. This is contrary to our original hypothesis. The outcome of our experiment may have been influenced by the planting design with a high planting density (1× 1 m). In our fine-grained mixture (1:1:1), the two fast-growing species dominated the upper canopy and clearly suppressed the *F. sylvatica* in the understory. The dense planting removed the need for extensive weed control. However, typical management techniques such as thinning may be needed to reduce competition and promote possible positive interactions in polyculture.

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Caption of the figures

Fig. 1 Experiment site and plantation design. a) 12 monoculture of *B. pendula* (Bi), *A. glutinosa* (Al), *F. sylvatica* (Be) and four three-species polyculture (M) plots, distributed over two plantation blocks. b) Sketch of hexagonal planting design in three species polyculture. Each plant was surrounded by six neighbours of two conspecific individuals, one and three of the other two species.

Fig. 2 Aboveground woody biomass in mono- and polyculture stands of *B. pendula*, *A. glutinosa* and *F. sylvatica* at the end of the 4th, 5th and 6th growing seasons. Woody biomass was estimated using species-specific allometric equations, developed by the tree harvesting approach after six years of stand age. Bars mean SE (n = 4). Within one year, bars without the same indices are significantly different ($P < 0.05$).

Fig. 3 Mixture effects in three species polyculture (positive and negative values indicate higher and lower biomass in polyculture than monoculture respectively). A) Species level differences in biomass. *F. sylvatica* showed significant mixture effect (-ve differences) but *A. glutinosa* and *B. pendula* had insignificant impacts on biomass. B) Stand-level mixture effect. **T**he differences between measured (in polyculture stand) and predicted (calculated from monoculture stands) woody biomass, which were not statistically significant. Bars mean SE (n= 4), * indicates statistically significant differences.

Fig. 4 Relationships between soil C stocks and : a) Aboveground woody biomass b) annual litter fall and c) fine root (< 2 mm) biomass. Soil organic C stocks were measured in 16 mono- and polyculture stands in 0-50 cm depth.

Appendix Fig. S1 Allometric relationship between plant variables and woody biomass (WB) of *B. pendula*, *A. glutinosa* and *F. sylvatica*. Scattered plots show data from 10 selected plants and the best fitted lines with basal diameter for *B. pendula* and *F. sylvatica* and diameter at breast height (DBH) for *A. glutinosa*. The regression equations, coefficient of determination and *P* values (using ANOVA) are inserted in each panel.

Table 1 Biometric data and plot size of different tree species in mono and polyculture plantation. Tree height, DBH (at 1.3 m above the ground level), basal diameter at 22.5 cm and basal area were estimated in both mono and polyculture plots. In polyculture plot, basal area of individual species was calculated separately. Mean, ± SE. To compare between mono and polyculture, data with similar alphabet are not statistically significant.

Parameter	***B. pendula***		***A. glutinosa***		***F. sylvatica***	
	Monoculture	**Polyculture**	**Monoculture**	**Polyculture**	**Monoculture**	**Polyculture**
Tree height (m)	5.71 ± 0.30a	5.83 ± 0.32a	6.18 ± 0.46a	6.18 ± 0.33a	3.19 ± 0.14a	2.10 ± 0.06b
DBH (mm)	41.79 ±2.87a	44.75 ± 4.90a	50.77 ± 2.68a	57.58 ± 3.53a	23.91 ± 1.65a	9.53 ± 0.59b
Basal diameter (mm)	58.67 ± 3.77a	63.21 ± 7.70a	67,33 ± 2.05a	76.26 ± 2.72b	36.33 ± 1.80a	18.87 ±0.78b
Basal area (m^2ha^{-1})	20.27 ± 3.42a	22.19 ± 4.72a	25.24 ± 1.66a	36.56 ± 5.18a	6.08 ± 0.42a	1.15 ± 0.19b
Number of tree $plot^{-1}$	87 ± 10	65 ± 5	84 ± 5	65 ± 5	80 ± 2	65 ± 5
Plot size (m^2) (n=4)	74 ± 15	156 ± 9	82 ± 13	156 ± 9	68 ± 4	156 ± 9

Table 2 Allometric equations for *B. pendula*, *A. glutinosa* and *F. sylvatica* to estimate woody biomass. General power model $y = a x^b$ was used, where y = woody biomass (kg) of plant, x = tree variables, i.e. D, d, b and h denotes DBH (at 1.3 m above the ground level), basal diameter (at 22.5 cm), branch dry weight and tree height respectively, a and b are regression coefficients.

Plant Species	Tree variables	Equations ($y = a x^b$)	r^2	F	*P*	RMSE*
B. pendula	DBH (mm)	$Y = 0.0008\ D^{2.2322}$	0.9938	1285.70	< 0.0001	0.3459
	Basal diameter (mm)	$Y = 0.0002\ d^{2.3893}$	0.9970	2746.41	< 0.0001	0.2370
	Branch dry weight (kg)	$Y = 4.4302\ b^{0.7502}$	0.9345	114.18	<0.0001	1.1256
	Tree height (m)	$Y = 0.0001\ h^{5.8014}$	0.8773	57.227	< 0.0001	1.5406
A. glutinosa	DBH (mm)	$Y = 0.0006\ D^{2.2775}$	0.9946	1491.32	< 0.0001	0.3807
	Basal diameter (mm)	$Y = 0.0001\ d^{2.6453}$	0.9884	682.81	< 0.0001	0.5610
	Branch dry weight (kg)	$Y = 6.3385\ b^{1.2229}$	0.9297	105.93	< 0.0001	1.3815
	Tree height (m)	$Y = 0.0048\ h^{3.5841}$	0.7898	30.07	< 0.0006	2.3900
F. sylvatica	DBH (mm)	$Y = 0.0071\ D^{1.6883}$	0.9836	480.28	< 0.0001	0.2151
	Basal diameter (mm)	$Y = 0.0002\ d^{2.5770}$	0.9847	517.46	< 0.0001	0.2073
	Branch dry weight (kg)	$Y = 2.8883\ b^{0.8845}$	0.841	42.32	< 0.0002	0.6706
	Tree height (m)	$Y = 0.0396\ h^{2.8864}$	0.7189	20.47	< 0.0019	0.8912

*RMSE, root mean square error.

Table 3 Fine root (<2mm diameter) biomass, production and turnover of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica* grown in single and mixed species plots. Fine root production during the growing season (June–November) was estimated by a root mesh technique. In polyculture, the mesh was deployed to capture the root growth from specific trees (not overall). Shown are mean ± SE, (n = 4). Values vertically with similar indices are not statistically significant (P<0.05).

Plantation plots (n=4)	**Fine root biomass at different soil depths (g m^{-2})**				**Fine root production (g m^{-2})**		**Fine root turnover (rate y^{-1})**
	0-10 cm	**10-20 cm**	**20-30 cm**	**Total**	**Single plot**	**Mixed plots**	
B. pendula	31.5 a ±2.9	25.8 a ±4.5	12.1 a ±4.0	69.4 ab ±8.3	55.8 a ±19.2	58.9 ab ±8.1	1.1 a ±0.5
A. glutinosa	38.4 a ±4.8	35.6 a ±3.8	17.0 a ±1.7	91.0 a ±2.9	72.1 a ±6.0	83.9a ±10.5	1.0 a ±0.2
F. sylvatica	21.9 a ±2.9	24.7 a ±6.5	12.7 a ±2.4	59.3 b ±8.7	26.6 b ±6.6	30.1 b ±5.6	0.6 a ±0.2
Polyculture	27.1 a ±3.0	33. 6a ±7.0	19.7 a ±4.5	80.4 ab (measured) ±12.7 73.2 ab (predicted)* ±10.9	-	-	1.1 a ±0.2

***** Total predicted value for mixed plot was estimated from total values of single species plots.

Table 4 Soil C and N stocks in monoculture stands of different tree species and a polyculture stand. The vertical distribution of stocks are shown for each 10 cm soil layer. Values equal mean ± SE (n=4). Values with different lowercase letters indicate statistically significant variation among four forest types ($p<0.05$).

Soil depth (cm)	Soil C stock (kg m^{-2})				Soil N stock (g m^{-2})			
	B. pendula	***A. glutinosa***	***F. sylvatica***	**Polyculture**	***B. pendula***	***A. glutinosa***	***F. sylvatica***	**Polyculture**
0-10	2.38 ±0.28**a**	2.04 ±0.09ab	1.59 ±0.23**b**	2.11 ±0.17ab	234 ±10a	225 ±8a	212 ±18a	226 ±11a
10-20	1.65 ±0.07**a**	1.56 ±0.10**a**	1.17 ±0.12**b**	1.36 ±0.09ab	185 ±13a	181 ±9a	157 ±10a	174 ±15a
20-30	1.03 ±0.09a	1.11 ±0.08a	0.94 ±0.16a	0.85 ±0.09a	133 ±9a	156 ±10a	137 ±14a	128 ±12a
30-50	1.26 ±0.14a	1.21 ±0.12a	0.91 ±0.04a	1.06 ±0.10a	182 ±17a	199 ±**3a**	183 ±37a	164 ±15a
Total (0-50 cm)	6.32 ± 0.48**a**	5.92 ±0.28a	4.61 ±0.37a	5.38 ±0.13a	734 ±21a	744 ±32a	689 ±51a	692 ±33a

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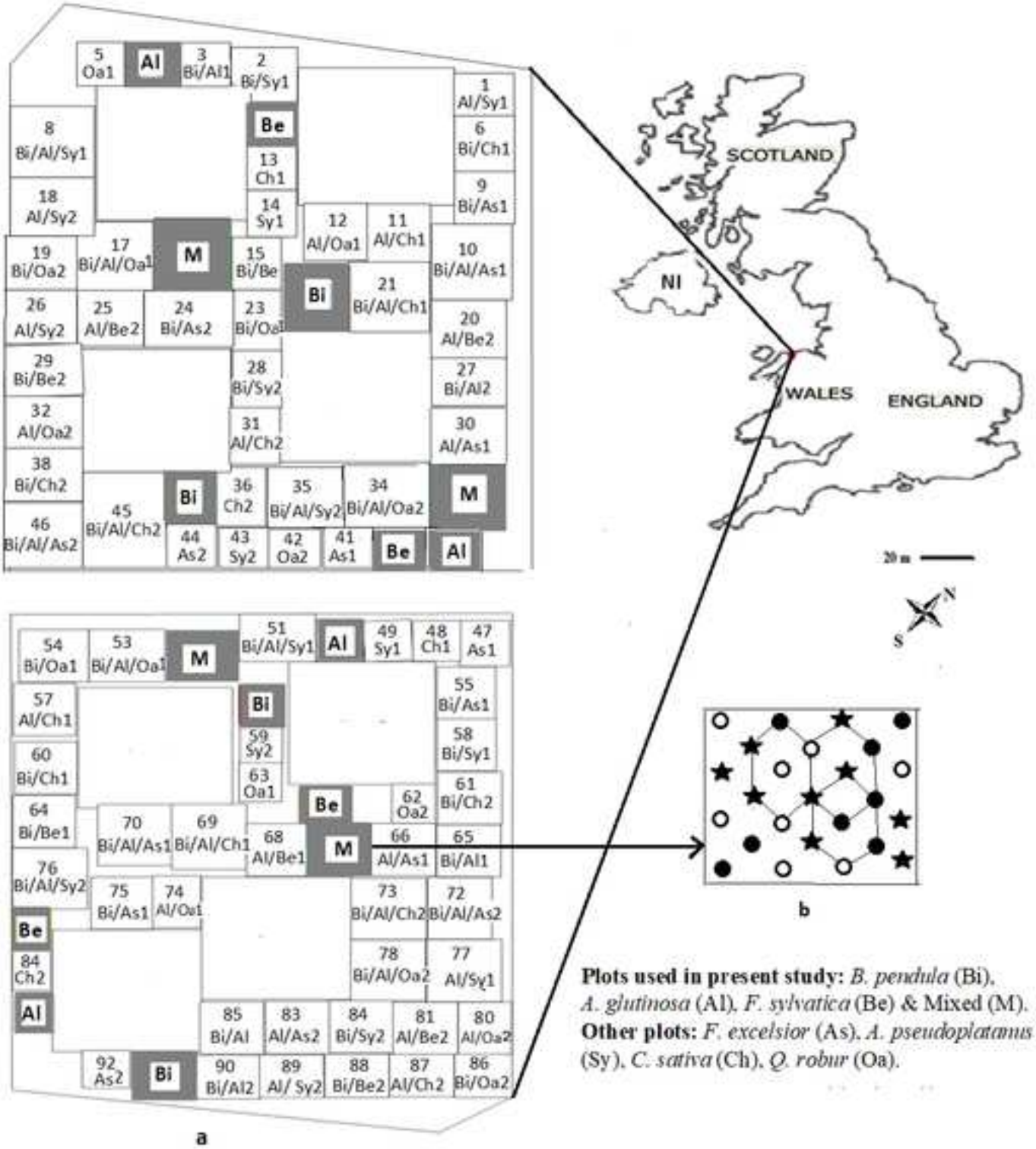

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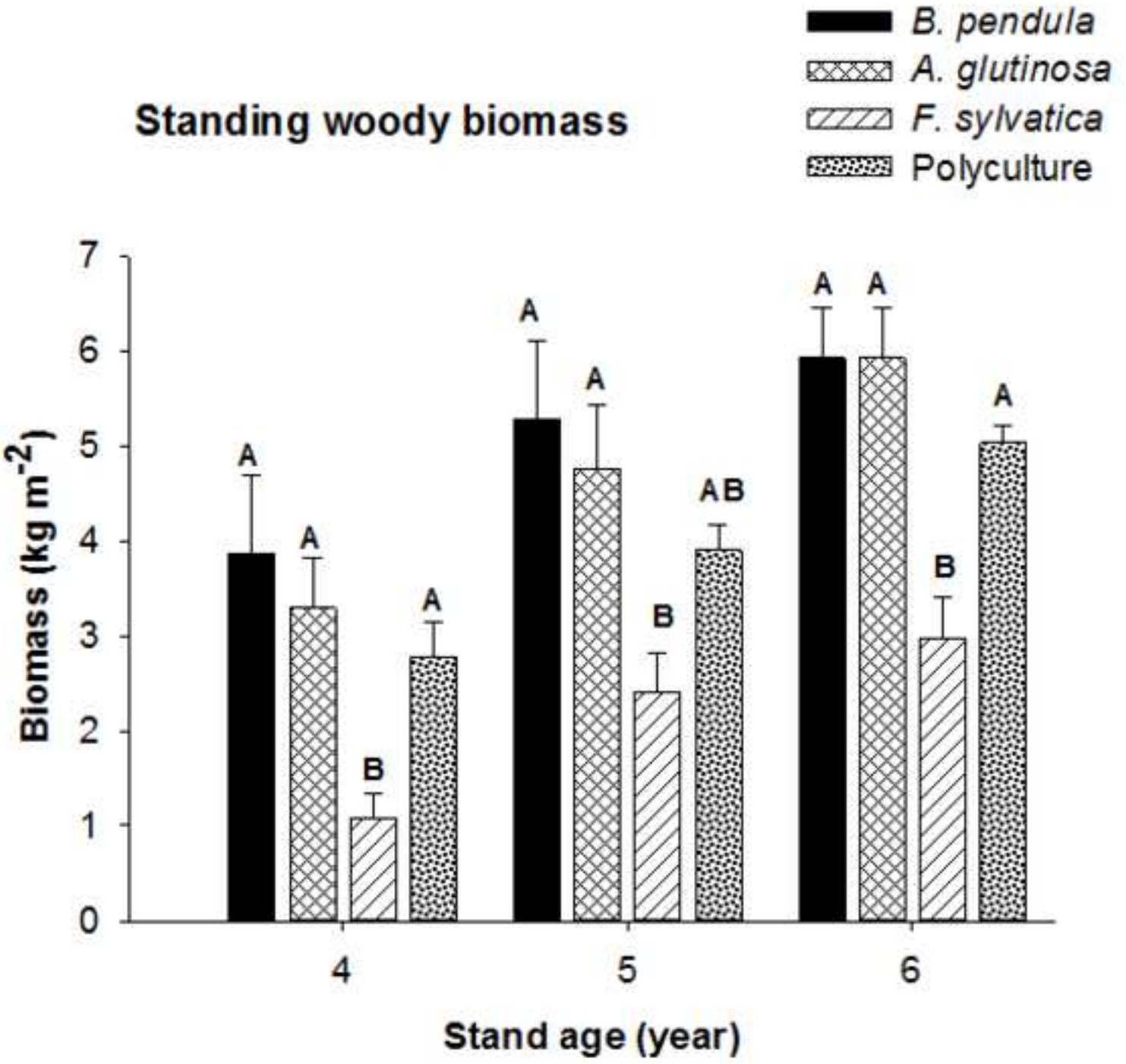

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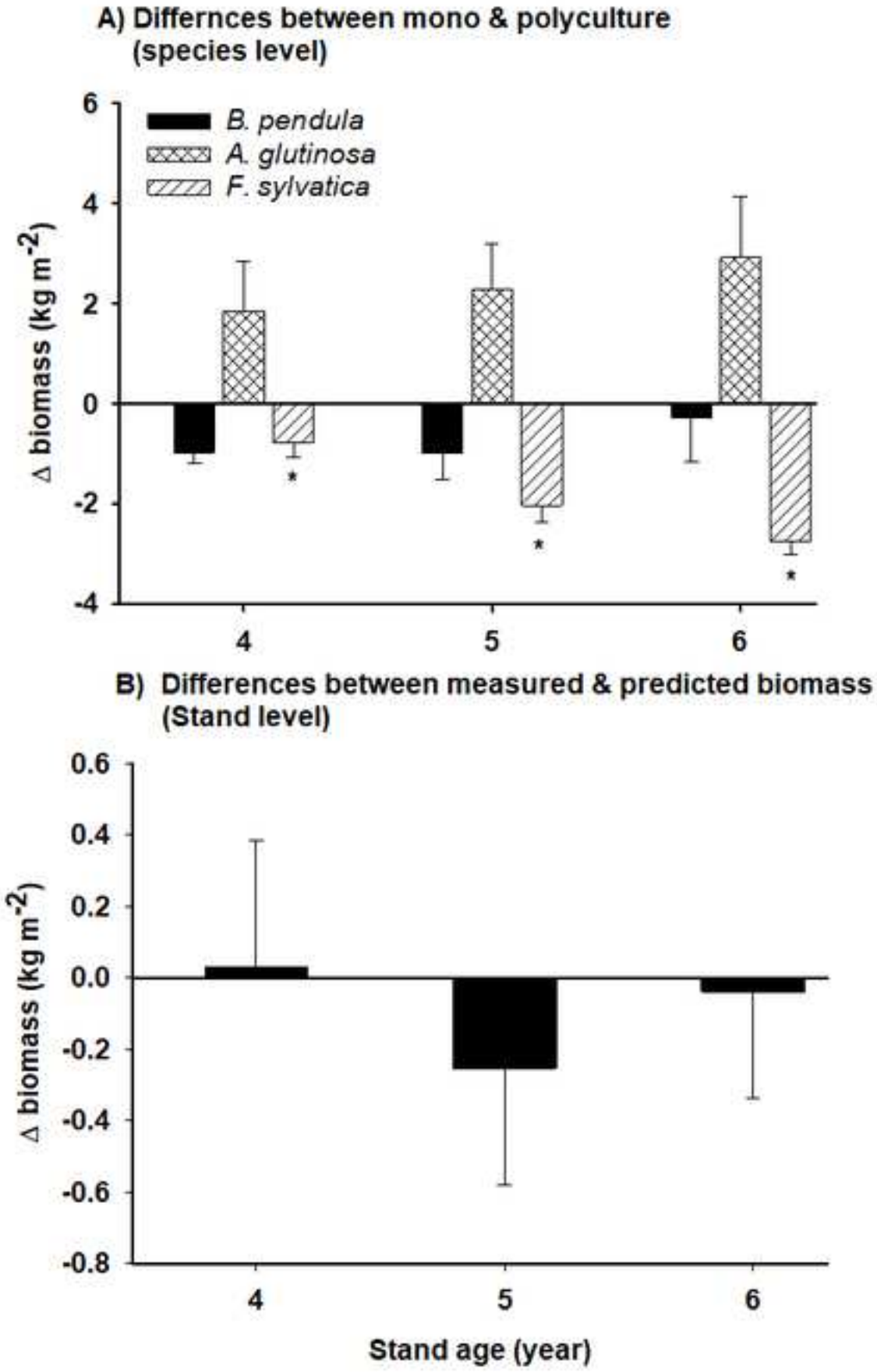

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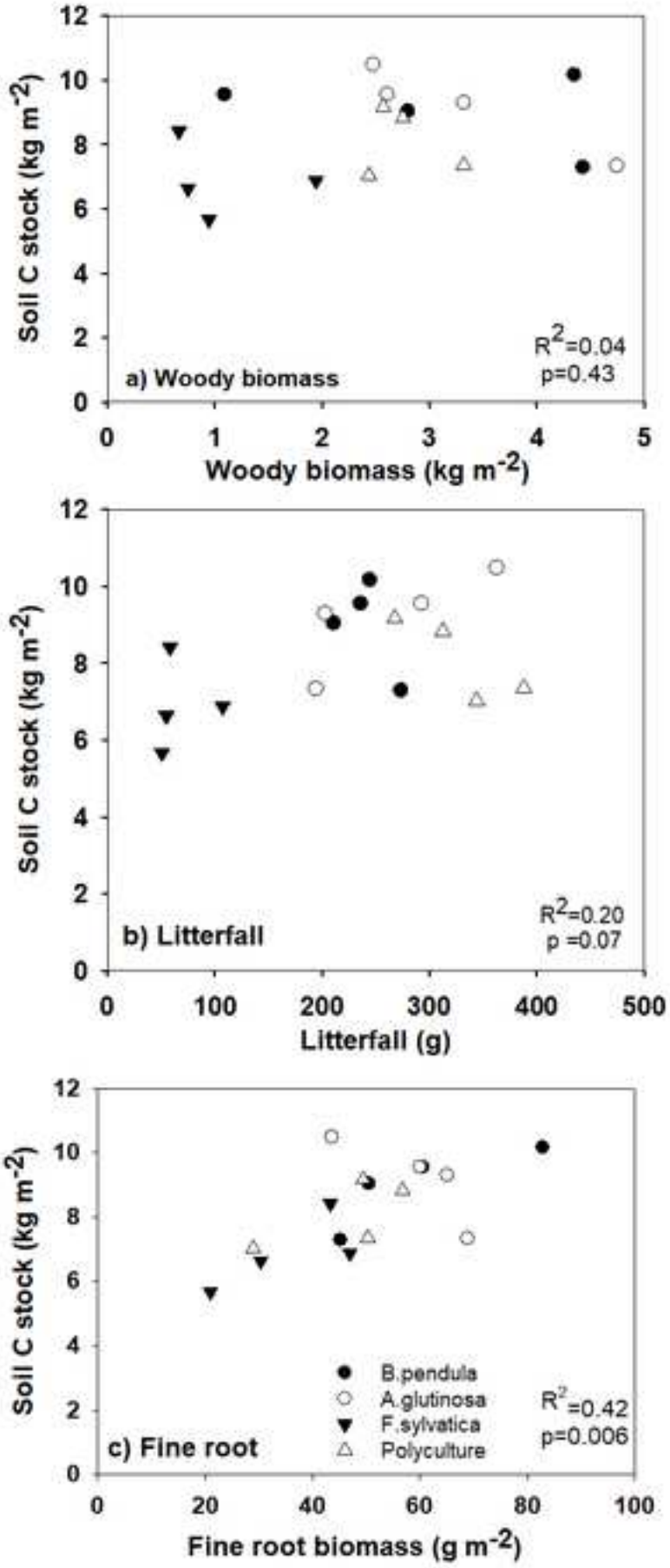

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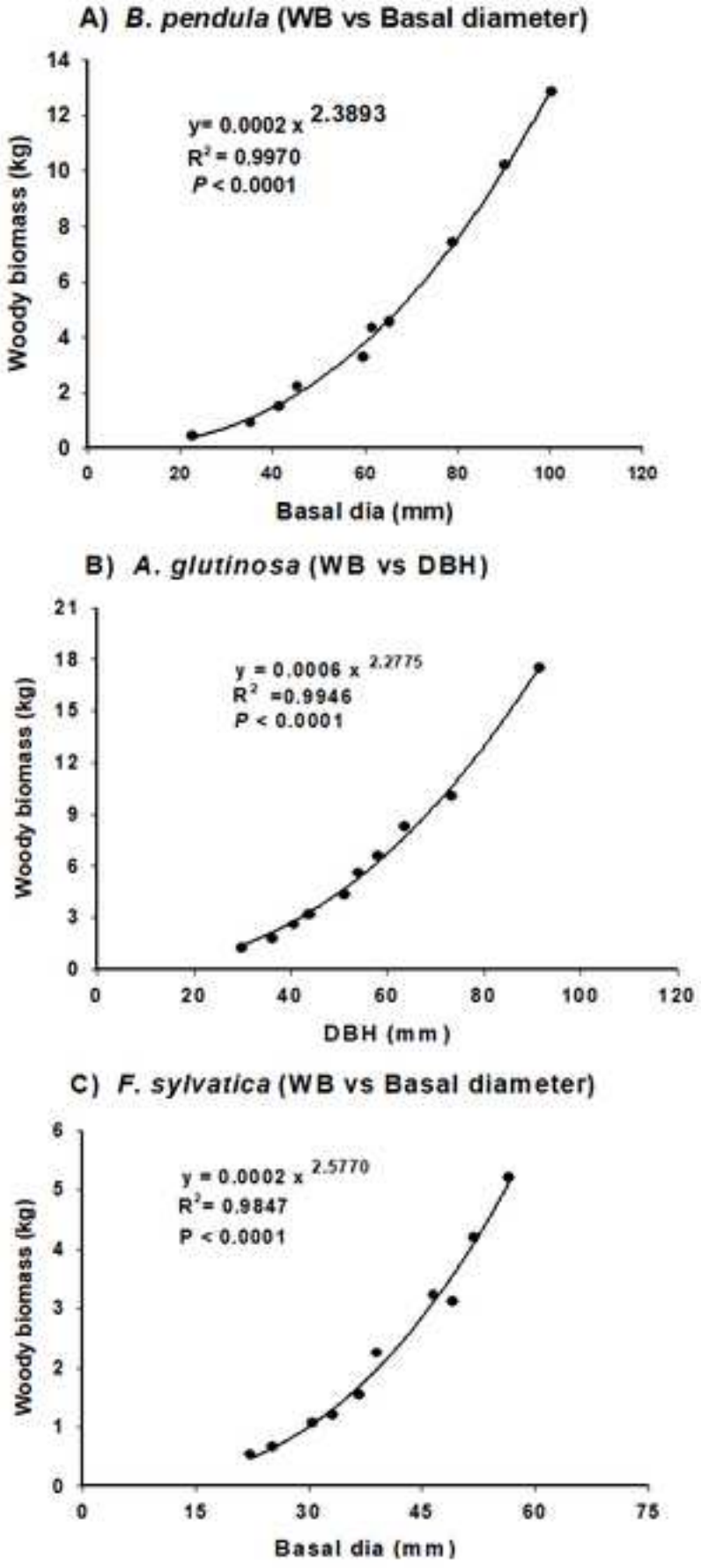

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Appendix Table A1 Exponential and logarithm models to examine the relationship between woody biomass and DBH and basal diameter (diameter at 22.5 cm). General model $y = a\, e^{bx}$ (exponential) and $y = y_0 + \ln x$ (Logarithm), where y = woody biomass of plant (kg), x = tree variables (here D and d denotes DBH and basal dia. (diameter) in mm respectively), a and b are regression coefficients.

Plant Species	Tree parameter	Equation types	Equations	R^2	F	*P*	RMSE*
B. pendula	DBH	Exponential	**$Y = 0.6242\, e^{0.0407D}$**	0.9757	321.47	< 0.0001	0.6854
		Logarithm	**Y = -22.0148 +7.2838 ln D**	0.7744	27.47	< 0.0001	2.0891
	Basal dia.	Exponential	**$Y = 0.5264\, e^{0.0323d}$**	0.9831	467.47	<0.0001	0.5706
		Logarithm	**Y =-27.6937 + 8.0970 ln d**	0.7964	31.30	0.0005	1.9845
A. glutinosa	DBH	Exponential	**$Y = 0.8275\, e^{0.0337D}$**	0.9744	304.64	< 0.0001	0.8340
		Logarithm	**Y = -48.0301+13.7332 ln D**	0.8775	57.34	< 0.0001	1.8243
	Basal dia.	Exponential	**$Y = 0.5916\, e^{0.0304d}$**	0.9673	273.06	< 0.0001	0.9420
		Logarithm	**Y = -63.4545+ 16.4406 ln d**	0.8733	55.16	< 0.0001	1.8555
F. sylvatica	DBH	Exponential	**$Y = 0.4777\, e^{0.0488D}$**	0.9485	147.59	< 0.0001	0.3811
		Logarithm	**Y = -10.1198+ 3.7605 ln D**	0.9457	139.36	< 0.0001	0.3917
	Basal dia.	Exponential	**$Y = 0.1842\, e^{0.0595d}$**	0.9807	407.38	< 0.0001	0.2332
		Logarithm	**Y = -14.9092+ 4.7496 ln d**	0.8823	59.98	< 0.0001	0.5764

* RMSE, root mean square error

Appendix Table A2**:** Soil physico-chemical properties at the experiment site. Values mean ±SE (n=4).

Soil Depth (cm)	**pH (H_2O)**	**Electrical conductivity (EC) (µS cm^{-1})**	**% soil fractions**			**Textural Class**	**Bulk density (gcm^{-3})**	**Soil organic matter (LOI)* %**	**C:N ratio**
			Sand	**Silt**	**Clay**				
0-10	5.41 ±0.07	130.5 ±11.4	48.1 ±1.4	33.6 ±0.9	18.3 ±2.2	Loam	1.19 ±0.01	6.46 ±0.46	9.2
10-20	5.6 ±0.1	67.2 ±6.1	49.3 ±2.3	33.1 ±0.6	17.6 ±2.1	Loam	1.29 ±0.02	5.74 ±0.43	8.1
20-30	5.8 ±0.1	48.9 ±3.93	49.5 ±2.47	33.4 ± 0.56	17.2 ±2.05	Loam	1.33 ±0.05	4.76 ±0.38	7.2
30-40	5.9 ±0.04	38.9 ±3.5	49.4 ±3.1	34.8 ±0.9	15.8 ±2.3	Sandy Loam	1.40 ±0.08	3.84 ±0.37	6.7
40-50	6.0 ±0.1	36.0 ±3.5	51.7 ±4.8	32.3 ±2.5	15.9 ±2.6	Sandy Loam	1.47 ±0.11	3.56 ±0.29	6.0

***Loss on ignition method**

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Appendix Table A3: Dry mass and C content in of different components of forest litter. Data show the annual quantity of litter fall (June –May). In polyculture plots quantity includes mixture of three species as collected in the traps. Values equal mean, SE (n=4).

Plantation plot	Different component of litter (g m^{-2}) and C content												
	Leaves	% C	% N	Branch	% C	% N	Twigs	%C	% N	Catkins	% C	%N	Total litter (g m^{-2})
B. pendula	323 ±31	52	1.84	15.2 ±7.6	55	1.08	1.2 ±0.1	54	ND	2.0 ±0.5	58	2.2	342 ±39
A. glutinosa	349 ±30	54	3.28	10.4 ±2.1	53	1.13	26.4 ±10.4	55	1.64	86.0 ±21.9	54	2.03	472 ±64
F. sylvatica	178 ±20	40	2.62	NF*	ND**	NF	NF	ND	NF	NF	ND	NF	178 ±20
Polyculture	326 ±37	53	2.97	22.9 ±2.3	54	1..36	17.7 ±1.0	ND	1.16	46.4 ±11.3	ND	2.13	413 ±51

*NF, Not found, **ND, Not determined.