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Elevated CO₂ enrichment induces a differential biomass response in a mixed species temperate forest plantation

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Summary

- In a free-air CO₂ enrichment study (BangorFACE) *Alnus glutinosa*, *Betula pendula* and *Fagus sylvatica* were planted in areas of one, two and three species mixtures (*n*=4). The trees were exposed to ambient or elevated CO₂ (580 µmol mol⁻¹) for four years, and aboveground growth characteristics measured.

- In monoculture, the mean effect of CO₂ enrichment on aboveground woody biomass was +29, +22 and +16% for *A. glutinosa*, *F. sylvatica*, and *B. pendula* respectively. When the same species were grown in polyculture, the response to CO₂ switched to +10, +7 and 0%, for *A. glutinosa*, *B. pendula*, and *F. sylvatica* respectively.

- In ambient atmosphere our species grown in polyculture increased aboveground woody biomass from 12.9 ± 1.4 kg m⁻² to 18.9 ± 1.0 kg m⁻², whereas in an elevated CO₂ atmosphere aboveground woody biomass increased from 15.2 ± 0.6 kg m⁻² to 20.2 ± 0.6 kg m⁻². The overyielding effect of polyculture was smaller (+7%) in elevated CO₂ than in an ambient atmosphere (+18%).

- Our results show that the aboveground response to elevated CO₂ is significantly affected by intra- and inter-specific competition, and that elevated CO₂ response may be reduced in forest communities comprised of tree species with contrasting functional traits.

keywords:
Free-air CO₂ enrichment (FACE), temperate forest, alder (*Alnus glutinosa*), silver birch (*Betula pendula*), European beech (*Fagus sylvatica*), allometry, overyielding, polyculture
Introduction

Forests occupy one third of the land surface of the Earth, and account for almost half of carbon stored in the terrestrial biosphere (Schlesinger & Lichter, 2001). In a summary of studies conducted to investigate the effects of increased atmospheric CO$_2$ on forest C cycles, Norby et al., (2005) calculated that an enrichment of 200 ppm CO$_2$ above the current ambient CO$_2$ level caused a 23% median increase of forest net primary productivity. However, interactions with other environmental factors may dampen such response at larger temporal or spatial scales (Leuzinger et al., 2011). Nevertheless, increasing atmospheric CO$_2$ concentrations may fundamentally alter forest ecosystem functioning by altering species growth, resource use and community interactions (Eamus & Jarvis, 1989). As forests are inextricably linked to the global carbon cycle, elevated CO$_2$ driven environmental change may impact upon global carbon storage in phytomass, complex biogeochemical feedback mechanisms and ultimately long term C sequestration in soils.

Empirical studies on woody plants exposed to elevated atmospheric CO$_2$ have demonstrated that growth and aboveground biomass production in woody plants increases, but that there is a considerable variation in response (Curtis & Wang, 1998). The observed variation of responses to elevated CO$_2$ has been attributed to a large number of confounding factors, such as the length of study, interactions with other environmental stresses, plant functional group, species morphological physiology (Poorter, 1993), symbiotic associations (Godbold et al., 1997) and community dynamics (Kozovits et al., 2005). Recent research efforts have been focused on studying whole ecosystem responses in near-natural conditions chiefly achieved by employing Free Air Carbon dioxide Enrichment (FACE) facilities (Hattenschwiler et al., 2002; Karnosky et al., 2003; Körner et al., 2005; Hoosbeek et
Körner (2006) has suggested dividing elevated CO₂ studies into the following two types: (i) high abundance of major resources other than carbon – ‘decoupled’ systems and (ii) near to steady-state nutrient cycling and full canopy development – ‘coupled systems’. Type I systems include the present study, aspen FACTS II FACE (Karnosky et al., 2003), and EuroFACE (Calfapietra et al., 2003) experiments. The remaining three (type II) experiments have used CO₂ enrichment in stands with an already closed canopy. The Oak Ridge (Norby et al., 2002) and DukeFACE (Oren et al., 2001) experiments both started enrichment ca. 10-20 years after planting, while at the Basel Web-FACE (Körner et al., 2005) enrichment was conducted in a mature deciduous forest comprised of four species more than 100 years old. Using data from four of these studies (DukeFACE, FACTS II FACE, Oak Ridge and EuroFACE), Norby et al., (2005) demonstrated that an enrichment of 200 ppm CO₂ above the current ambient CO₂ level caused a 23% median increase of forest net primary productivity. This conclusion was largely based on the initial response of forest ecosystems to elevated CO₂. Subsequent investigations have shown that this response may not be maintained over a longer time horizon (Norby et al., 2010), as the response to elevated CO₂ has been found to both decline (Norby et al., 2010) or be maintained (Drake et al., 2011; Zak et al., 2011) after 10-11 years of exposure. In both of these examples, the response to elevated CO₂ was likely mediated by N availability. The decline in response to elevated CO₂ was attributed to N limitation (Norby et al., 2010), while no change in response was a result of greater N cycling (Zak et al., 2011). Comparison of these two studies clearly demonstrates that nutrient availability, in particular N, is a strong factor mediating the response of woody plants to elevated CO₂.
Much of the research investigating species diversity, ecosystem functioning and productivity has been focused in grasslands (Hooper et al., 2005). Many experiments have shown a positive relationship between productivity and increased biodiversity (Tilman et al., 1996; Tilman et al., 1997). Fornara & Tilman (2009) suggested that the increased productivity of N-limited species rich plant communities is dependent on the seasonal accumulation of root N pools by N-fixing plants. The importance of incorporating N-fixing plants in the facilitation of greater plant community productivity was also supported by Hooper & Dukes, (2004), but argued that N-fixation is not the only mechanism explaining the overyielding of species rich communities. Elevated CO$_2$ has been found to stimulate symbiotic N fixation in several studies (eg. Hungate et al., 1999; Schortemeyer et al., 2002), and the incorporation of N-fixing plants to facilitate N dynamics of co-occurring species with elevated CO$_2$ was explored by Lee et al., (2003) who found that in nine different grassland species assemblages incorporating N-fixing Lupinus did not facilitate a larger community growth response to elevated CO$_2$.

In forests, controversy surrounding the benefits of mixed species stand productivity dates back to the 18th century (Hartig, 1791), with silvicultural practice of mixed species forests being subject to much conjecture. Only recently have rigorous scientific studies been initiated to elucidate the precise mechanisms mediating the productivity differences of trees grown in polyculture (Pretzsch, 2005). For example, in Southern Germany, mixed stands of Fagus sylvatica and Picea abies produced up to 59% more aboveground biomass than adjacent pure stands (Pretzsch & Schütze, 2009). In contrast, Jacob et al. (2010) found decreases in aboveground biomass of F. sylvatica with increasing species richness in comparison to F. sylvatica in monoculture. Early on, most research on forest diversity focused on one or two tree
species, but recent studies included more species in an attempted to verify the
applicability of grassland findings to forest stands (DeClerck et al., 2006; Vila et al.,
2007; Paquette & Messier, 2010). In large scale investigations, support has been
found for the assertion that increased tree diversity leads to increased biomass
production (Vila et al., 2007; Paquette & Messier, 2010). The studies of both Vila et
al., (2007) and Paquette & Messier (2010) used databases originating from national
forest inventories, while taking into account the effects of environment. Paquette &
Messier (2010) used 12,000 permanent forest plots in boreal and temperate forest in
Canada, and could show a strong positive and significant effect of tree biodiversity on
aboveground productivity. The study of Vila et al., (2007) used over 8,000 permanent
forest plots in mediterranean forests in Catalonia, and could show a mean 30% higher
wood production in mixed forest compared to mono-specific stands, and a production
increase from 23% in two species stands to 59% in five species stands. In a meta-
analysis of 54 forest studies investigating diversity–productivity relationships, Zhang
et al., (2012) could show a 24% higher productivity in polycultures than monocultures
with most of the variation accounted for by evenness, the heterogeneity of shade
tolerance, species richness and stand age, in decreasing order of importance. Recently,
high plant diversity has been shown to be required to maintain ecosystem function and
services through time (Isbell & Wilsey, 2011), however the role of tree diversity in
ecosystem productivity, resistance and resilience is still poorly investigated (DeClerck
et al., 2006). In the case of resistance to drought, DeClerck et al. (2006) found that the
relative percentage of different species was more important than the species richness
per se. Differing species resistance to drought can change the competitive relationship
between the species and may thus result in changed species composition. Reich et al.,
(2001) could show that the enhancement of biomass accumulation in response to
elevated levels of CO$_2$ was smaller in species-poor than in species-rich assemblages of herbaceous plants. However, although it has long been known that tree seedlings of co-occurring species show differing response to CO$_2$ (Bazzaz & Miao, 1993), the influence of elevated CO$_2$ on tree competition, and the influence of tree biodiversity on community response to CO$_2$ has not been investigated.

The objectives of this work were to investigate the effects of elevated CO$_2$ (580 µmol mol$^{-1}$) on the species and community response of monocultures and polycultures of tree mixtures under field conditions. Using a Free Air Carbon dioxide Enrichment (FACE) system we investigated the aboveground response of monocultures and a three species polyculture of *Alnus glutinosa*, *Betula pendula* and *Fagus sylvatica* to elevated CO$_2$ over four years. We tested the hypothesis that interspecific competition modifies the response of tree species to elevated CO$_2$.

**Materials and Methods**

*Site description*

The Bangor FACE experimental site was established in March 2004 at the Bangor University research farm (53°14′N, 4°01′W) on two former agricultural fields with a total area of 2.36 ha. Both fields were originally pastures, one field was used for small scale forestry experiments for the last 20 years, the other field was ploughed and planted with oil seed rape in 2003. Climate at the site is classified as Hyperoceanic with a mean annual temperature in 2005 through 2008 of 11.5 °C and an annual rainfall of 1034 mm. Soil parent material is postglacial alluvial deposits from the Aber river which comprises Snowdonian rhyolitic tuffs and lavas, microdiorites and dolerite in the stone fractions and Lower Paleozoic shale in the finer fractions. Soil is a fine loamy brown earth over gravel (Rheidol series) and classified as Fluventic Dystrochrept (Teklehaimanot *et al.*, 2002). Soil texture is 63% sand, 28% silt and 9%
clay, nitrogen content in the top 30 cm is 2.6% with C/N ratio of 10.5. The
topography consists of a shallow slope of approximately 1–2° on a deltaic fan. The
site aspect is northwesterly, with an altitude of 13 to 18 m a.s.l. The depth of the water
table ranges between 1 and 6 m.

Eight octagonal plots, four ambient and four CO$_2$ enriched were established at
the site, creating a 2 × 4 factorial block design across the two fields. We used three
tree species (*Alnus glutinosa* [L.] Gaertner, *Betula pendula* Roth. and *Fagus sylvatica*
L.) selected due to their contrasting shade tolerance, successional chronology and to
represent a range of taxonomic, physiological and ecological types. 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 inter-tree spacing of 0.8 m, giving a density of 15,000 tree ha$^{-1}$. A
systematic hexagonal planting design (Aguiar et al., 2001) was used to maximise the
mixing effect so that, in the three-species polyculture sub-plots, each tree was
surrounded by nearest neighbours of two-conspecific individuals and one and three
individuals of the other two species respectively, resulting in each tree having six
equidistant neighbours. Each plot was divided into seven planting compartments and
planted in a pattern creating areas of one, two and three species mixtures (Fig. 1). The
present study makes use of observations originating from three single species sub-
plots containing nine trees of *B. pendula, A. glutinosa* and *F. sylvatica*, and a fourth
sub-plot which contained a species balanced polyculture of all three species. The
planting pattern of each pair of control and elevated CO$_2$ plots was rotated by 90° to
avoid potential artefacts introduced by microclimate, soil and uneven growth rates of
the different species. Each plot was surrounded by a 10 m border of *B. pendula*, *A. glutinosa* and *F. sylvatica* planted at the same density. The remaining field was planted at a 1 m spacing (10,000 trees ha\(^{-1}\)) with a mixture of birch (*B. pendula*), alder (*A. glutinosa*), beech (*F. sylvatica* L.), ash (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanus* L.), chestnut (*Castanea sativa* Mill.) and oak (*Quercus robur* L.). To protect the saplings, the entire plantation was fenced.

Eight steel towers were erected around each plot to delineate the experimental area and to provide supporting infrastructure for the CO\(_2\) enrichment system in the treatment plots. Ambient CO\(_2\) control plots were identical to the treatment plots, but for the absence of CO\(_2\) injection piping, to ensure any infrastructure introduced artefacts were applied to both the treatment and control. Carbon dioxide enrichment was carried out using high velocity pure CO\(_2\) injection (Okada *et al*., 2001). In the first two growing seasons, CO\(_2\) was delivered from a horizontal pipe held at canopy level. In the growing seasons 3 and 4, an additional pipe suspended 2 m below the canopy pipe was added to provide adequate enrichment throughout the canopy.

Control of CO\(_2\) delivery was achieved using equipment and software modified from EuroFACE (Miglietta *et al*., 2001). The target concentration in the elevated CO\(_2\) plots was ambient plus 200 ppm. The elevated CO\(_2\) concentrations, measured at 1 minute intervals, were within 30% deviation from the pre-set target concentration of 580 ppm CO\(_2\) for 75-79% of the time during the photosynthetically active (daylight hours between budburst until leaf abscission) period of 2005 – 2008. Vertical profiles of CO\(_2\) concentration measured at 50 cm intervals through the canopy showed a maximum difference of +7% from reference value obtained at the top of the canopy.

The effect of CO\(_2\) fumigation on diameter and height of trees grown within the plots was not modified by the distance from the CO\(_2\) delivery pipe (Supporting Information...
Fig. S1). The CO$_2$ used for enrichment originated from natural gas and had a δ$^{13}$C of -39‰.

Biometric Measurements

Tree height and stem diameter at 22.5 cm were measured after tree establishment in March 2005 and then February of each following year during CO$_2$ enrichment (2006-2009). Tree measurements were taken during the winter dormant phase to prevent growth introduced variation. Tree height was determined using a telescopic pole, and two measurements of diameter were taken perpendicular to each other using digital vernier callipers. To account for elliptical stem shape a geometric mean was calculated. As the initial tree height was less than 137 cm it was only possible to measure diameter at breast height (DBH) in subsequent years as the stand developed.

Allometric Relationships, Stem Volume Index

Two trees of each species were selected for destructive harvest from the downwind buffer zone of each treatment and control plot. The selection of trees for each species was based on average height and diameter data collected during the previous season. Tree height and stem diameter at 22.5 cm were measured and the trees were excavated to a root diameter of 3-4 mm then separated into leaves, branches stems and roots. Roots were washed free of adhering soil and stems cut into 15-20 cm sections, oven dried at 80 °C for 72 hrs and weighed. As a consequence, a power regression of stem diameter and woody biomass was used to explain the allometric relationship for each species studied since height was not found to contribute significantly to any of the allometric models tested (Equation 1). Equation 2 shows the biomass allometric
equation in its linear form. Where $D$ is stem diameter at 22.5 cm, with the power regression scaling coefficients $a$ (amplitude) and $b$ (exponent).

$$biomass = aD^b$$

Eqn 1

$$\ln(biomass) = \ln(a) + b \ln(D)$$

Eqn 2

Stem volume index (basal diameter$^2 \times$ height) was calculated and correlated against allometrically determined biomass to test the accuracy of predicted biomass values.

Overyielding

To determine the effect of growing species in polyculture, the total measured aboveground woody biomass values in the three-species polyculture sub-plots was compared with a theoretical mixture calculated from the biomass of each species growing in the monoculture sub-plots. Equation 3 shows the theoretical mixture biomass calculation based on the stem number contribution of each species to the polyculture, where $B_{Species}$ is the biomass component contributing to 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 3 in this experiment is comparable with the Relative Yield Total (Weigelt & Jolliffe, 2003).

$$B_{mixture} = \left( \frac{1}{3} \times B_{Ahnus} \right) + \left( \frac{1}{3} \times B_{Betula} \right) + \left( \frac{1}{3} \times B_{Fagus} \right)$$

Eqn 3
Leaf N contents

Leaf N contents were measured on five fully mature but otherwise unaltered leaves collected throughout the canopy of each species sub-plot (120 leaves in total) in 2006 (Ahmed, 2006), 2007 (Anthony, 2007), and 2008 (Millett et al., 2012).

Leaf Area Index

From the beginning of leaf senescence, fallen leaf litter was collected weekly using litter baskets with an area of 0.11 m² until all leaves had abscised (October to December). A litter basket was located in each of the monoculture sub-plots and the three species polyculture sub-plot (4 in each experimental plot). Litter was washed in a laboratory, sorted by species and then dried at 80 ºC for 24 hours. Dry weight of each species was determined and recorded for each species sub-plot. Juvenile Fagus sylvatica was excluded from the calculations as the beech trees retained the foliage until bud burst the following season. Leaf area index was calculated according to (McCarthy et al., 2007). The specific leaf area was calculated from fresh leaves collected during 2006 and dried archived leaves collected in 2007. Measurements of leaf area were made with a LI 3000A portable area meter (LI-COR, Lincoln, NE, USA). Immediately following area measurement leaves were dried at 80 ºC for 24 hours, and weighed to determine specific leaf area. The LAI values obtained were then scaled to calibrate for the different number of trees per species per ground area in the monoculture and polyculture plots.

Statistical Analysis

Regression fitting was conducted using SigmaPlot v11.0 (Systat Software Inc, Chicago, IL.). All statistical procedures were undertaken with SPSS 17.0 (SPSS Inc.,
Chicago, IL) with $P<0.05$ used as the limit for statistical significance. To avoid psuedoreplication the mean woody biomass per unit area (g m$^{-2}$) was calculated from the trees contributing to the single and mixed-species plots and data were subjected to repeated measures ANOVA for time series analyses using the plots as replicates ($n=4$); equality of variance was tested using Mauchly’s test of sphericity. A General Linear Model was used to calculate univariate analysis of variance for data determined at conclusion of the experiment. Data were tested for normality using Shapiro-Wilk’s test and homogeneity of variance was determined using Levene’s test. Diameter distributions were compared by fitting a normal distribution into the frequency data and testing for differences in the peak diameter by extra sum-of-squares F test.

Results

Stem diameter and tree height

At the conclusion of the experiment, the treatment effect on diameter was most pronounced in single species sub-plots with the largest effect of +14% observed in *A. glutinosa* (ambient 49.1 mm, elevated CO$_2$ 55.9 mm, $P=0.007$, Table 1). Elevated CO$_2$ did not change stem diameter of *B. pendula* or *F. sylvatica* significantly. We assessed the treatment effects on diameter distributions of all species by grouping all measured trees into ten diameter classes with 10 mm step increment. For *A. glutinosa*, *B. pendula* and *F. sylvatica*, the most frequent diameter class was 50-60 mm, 40-50 mm and 20-30 mm, respectively. The diameter class distribution of *B. pendula* and *F. sylvatica* grown in monoculture was not altered by elevated CO$_2$ enrichment (Supporting Information Fig. S2). However in *A. glutinosa*, there was a
shift towards larger diameter boles under elevated CO$_2$, where 39% of trees had a
diameter greater than 50-60 mm, which was in contrast to ambient plots, where only
11% of trees were in this diameter class ($P=0.021$). In polyculture, the mean of the
diameter distribution was not altered by elevated CO$_2$ in any of the species. Tree
height was unaffected by elevated CO$_2$ enrichment in either mono- or polyculture at
the end of observation (Table 1).

Allometric Equations

Height and diameter data gathered from trees in the vicinity of elevated and ambient
CO$_2$ plots were subjected to a stepwise biomass prediction regression. Height was
excluded during this analysis, as it did not significantly contribute to the regression
model. Ultimately a simple power regression of diameter predicted biomass with the
greatest accuracy. Power function scaling coefficients for the three species utilised in
this study are shown in Table 2. There were no changes in allometry due to elevated
CO$_2$ at this stage of tree development and subsequently all species specific data were
pooled to produce three allometric relationships with coefficients of variation ranging
from 0.78 to 0.85. Strong correlations between stem volume index and predicted
biomass confirmed the accuracy of predictions for A. glutinosa ($R^2=0.98$) and B.
pendula ($R^2=0.99$), but highlight a small underestimate of predicted F. sylvatica
biomass in elevated CO$_2$ plots ($R^2=0.88$).

Aboveground biomass in monoculture and polyculture.

Making use of the allometric equations to calculate tree aboveground woody biomass,
we show that species grown in monoculture responded to elevated CO$_2$ treatment
more than those grown in the three species polyculture. Fig. 2 and Table 3 detail the
relationship between time and biomass accrual for all species in ambient and elevated atmospheric CO$_2$. Under ambient CO$_2$ both *A. glutinosa* and *B. pendula* accumulated aboveground woody biomass faster in the polyculture than in the monocultures. The influence of elevated CO$_2$ on aboveground woody biomass production varied between species and years. Unsurprisingly in an expanding system, sampling year explained the greatest amount of variation in a repeated measures ANOVA model, being highly significant for all species in both monoculture and polyculture (Table 4). There were no significant year $\times$ treatment interactions for any species in the polyculture or for *B. pendula* and *F. sylvatica* in the monocultures. However, there was a significant year $\times$ treatment interaction for *A. glutinosa* ($P=0.008$). Elevated CO$_2$ treatment produced a significant effect on aboveground woody biomass in *A. glutinosa* grown in monoculture during 2005 ($P=0.022$), 2007 ($P=0.025$) and 2008 ($P=0.002$, Table 3). In polyculture, no statistically significant effects of elevated CO$_2$ were found.

The temporal fluctuation in the treatment effect of *B. pendula* and *F. sylvatica* grown in monoculture and polyculture became more apparent when the aboveground woody biomass NPP for each year was calculated (Table 5). In the monocultures, *A. glutinosa* showed a positive treatment effect throughout the 4 years of enrichment, whereas in *B. pendula* both positive and negative treatment effects were found. In *F. sylvatica*, aboveground woody biomass NPP was initially stimulated under elevated CO$_2$, but the effect turned strongly negative in 2008. In polyculture, *A. glutinosa* showed a strong positive treatment effect on aboveground woody biomass for all years except 2007. Similarly in *B. pendula* a positive treatment effect on aboveground woody biomass were shown for all years. In contrast, a negative effect of elevated CO$_2$ was shown on the accumulation of aboveground woody biomass in *F. sylvatica*. 
in all years except 2006. Pooling the species contributing to the polyculture over all years, there was no effect of elevated CO$_2$ on overyielding in the mixture ($P=0.094$), nor did we observe any modification of the CO$_2$ fertilisation when growing trees in monoculture or polyculture ($P=0.192$, Fig. 3).

At the conclusion of the experiment with all species pooled, aboveground woody biomass reached 16.5 ± 0.8 kg m$^{-2}$ in ambient CO$_2$ plots and 19.3 ± 0.4 kg m$^{-2}$ in elevated CO$_2$ plots, a significant increase of 17% ($P=0.022$). The contribution of aboveground woody biomass within the elevated CO$_2$ plots followed the order B. pendula (10.1 ± 0.0 kg m$^{-2}$), A. glutinosa (8.6 ± 0.6 kg m$^{-2}$) and F. sylvatica (0.6 ± 0.0 kg m$^{-2}$). A significant 16% ($P=0.046$) increase in aboveground woody biomass was observed in B. pendula in response to CO$_2$ treatment. Pooling the values for each species, in the monocultures the aboveground woody biomass was 12.9 ± 1.4 kg m$^{-2}$ in ambient, and 15.2 ± 0.6 kg m$^{-2}$ in elevated CO$_2$ treatments. Polyculture aboveground woody biomass reached 18.9 ± 1.0 kg m$^{-2}$ in ambient and 20.2 ± 0.6 kg m$^{-2}$ in elevated CO$_2$ treatments. This resulted in an increase in aboveground woody biomass under elevated CO$_2$ of 18% in monoculture and 7% in polyculture.

To summarise, pooled aboveground woody biomass was significantly affected by elevated CO$_2$ ($P=0.022$). We also observed a significant positive effect of species mixture ($P=0.001$), but the interaction was not significant ($P=0.534$).

Leaf N content and aboveground NPP

Over the course of the experiment, leaf N contents were not significantly affected by elevated CO$_2$ (Table 6). However, we observed a strong increase in foliar N content in time ($P<0.001$), combined with significant differences between species ($P<0.05$) over the period 2006-2008 (Supporting Information Fig. S3). Leaf NUE, defined as unit of
aboveground NPP per unit of foliar N content (Yasumura et al., 2002), fluctuated in
time (Fig. 5) and was significantly increased by elevated CO$_2$ from 44.0 to 53.7 g m$^{-2}$
mg g$^{-1}$ averaged for all species and years ($P$=0.017). Due to data unavailability, we
could only establish the effect of mixture on leaf NUE in 2008. Four years into the
experiment, growing species in polyculture as opposed to monoculture significantly
increased the overall leaf NUE from 23.4 to 38.6 g m$^{-2}$ mg g$^{-1}$ ($P$=0.022, Fig. 6).
However, there was no effect of mixture or elevated CO$_2$ on leaf NUE in individual

Leaf Area Index
Repeated measures ANOVA showed a significant year × species interaction for
species grown in monoculture ($P$<0.05) and polyculture ($P$<0.001; Table 7). The
response of LAI to elevated CO$_2$ when species were grown in monoculture was a
mean increase of 32% in B. pendula, and mean decrease of 6% in A. glutinosa. During
the four years of CO$_2$ enrichment LAI of B. pendula was between 1.1-3.2 m$^2$ m$^{-2}$ in
ambient CO$_2$ and 0.8-4.0 m$^2$ m$^{-2}$ in elevated CO$_2$ plots, whereas LAI of A. glutinosa
was between 1.4-7.6 m$^2$ m$^{-2}$ and 1.4-8.2 m$^2$ m$^{-2}$ in ambient and elevated CO$_2$ plots
respectively (Fig. 4). Elevated CO$_2$ initially increased LAI of B. pendula by 37%,
however this effect gradually declined to 24% in 2007, recovering to 32% by the
conclusion of the experiment. In both mono- and polyculture, peak LAI in A.
glutinosa and B. pendula was recorded in 2007, which was preceded by a severe
drought, summer crown defoliation, and leaf re-flushing during august of 2006, a
strong decline in LAI immediately followed in 2008 in monocultures. During 2008 in
polyculture the LAI was 4.6 and 4.4 times greater than in monoculture in ambient
atmosphere for B. pendula and A. glutinosa, respectively, whilst in monoculture the
LAI was 6.1 and 4.6 times greater than in elevated CO₂ for B. pendula and A. glutinosa respectively.

Discussion

Allometric relationships have commonly been used to estimate biomass of aboveground compartments. The allometric coefficients generated in this study were broadly similar to previously published coefficients (Hughes, 1971; Bartelink, 1997; Pajtik et al., 2011), with the exception of F. sylvatica. The dimorphic growth characteristics of juvenile F. sylvatica under different light regimes during canopy development may explain the difference observed (Delagrange et al., 2006). The application of species and site specific allometric relationships is likely to be valid for A. glutinosa and B. pendula. However, the relationship for F. sylvatica appears a little weaker and may benefit from closer examination of the differences in morphology when trees are shade suppressed and growing in full light.

In this study, aboveground woody biomass accumulation in A. glutinosa and B. pendula was greater in polyculture than in the monocultures. In species diverse communities, complementary use of resources may lead to higher yields than in monocultures (Loreau & Hector, 2001). Differences in the tree species life-history character traits, such as crown structure, rooting depth, shade tolerance, phenology, and photosynthetic light response may allow for differential access to resources (Kelty, 1992). If the chosen species occupying the same site differ substantially in these characteristics, they may capture site resources more completely or use resources more efficiently to produce biomass. Species with contrasting trait characteristics can be described as having complementary resource use (Haggar & Ewel, 1997) or good ecological combining ability (Harper, 1977). However, it should
be noted that complementarity may not necessarily result in a positive effect on productivity, antagonistic interactions (negative complementarity) between species may also occur due to character trait interferences that may lower the productivity of species mixtures over those expected from monocultures (Wardle et al., 1998; Loreau & Hector, 2001; Eisenhauer, 2012). In this study, Paquette & Messier (2010) in an analysis of naturally occurring tree biodiversity could show a strong positive effect of biodiversity on tree productivity. They further suggest that in the more productive environment of temperate forest, competitive exclusion is the most probable outcome of species interactions, but in the more stressful environment of boreal forest beneficial interactions such as niche partitioning and facilitation may be more important.

In our temperate forest mixture, we used two pioneer species and a late successional species that strongly differ in their functional traits. *Betula pendula* is a light demanding, early successional pioneer species which casts little shade and rapidly occupies open areas due to fast juvenile growth (Fischer et al., 2002). *Alnus glutinosa* is an N-fixing, water demanding pioneer species, also with high juvenile growth rates (Braun, 1974). The root system of *A. glutinosa* is adapted to wet soils, with many vertically growing sinker roots that may reach 5 m depth (Claessens et al., 2010). In mixed forests, its limited height growth and shade intolerance prevent it from dominating in late successional forest. Lastly, *Fagus sylvatica* is shade tolerant and slow growing when juvenile (Ellenberg et al., 1991), can persist in the understory, and often dominates late successional forest. The higher polyculture productivity in our 4 year old plantation suggests that the dominant pioneer species *A. glutinosa* and *B. pendula* are partitioning canopy space made available by *F. sylvatica*. However, the flattening of the diameter class distribution in *B. pendula*, but not in *A. glutinosa*,
suggests that some *B. pendula* are being excluded. In our study, we did not systematically determine crown architecture, but observed that in polyculture both *B. pendula* and shorter *A. glutinosa* had deeper crowns. Indeed, we saw higher LAI in *A. glutinosa* and *B. pendula* in polyculture compared to monocultures, but no difference in stem height, which suggests alteration of crown architecture between monoculture and polyculture grown trees. Claessen *et al.* (2010) suggest that *A. glutinosa* grown in monoculture produces a straight bole and round crown, whereas when grown in admixture with other species forms a stratified canopy. In the meta-analysis of species richness productivity relationships by Zhang *et al.*, (2012), heterogeneity of shade tolerance was the second most important factor explaining increased productivity in mixtures. In addition to an aboveground partitioning of canopy space, an increase in N availability via the N-fixing *A. glutinosa* could also be a factor in the higher productivity of the polyculture. In *A. glutinosa* under ambient CO₂, the amount of N content in the leaves did not differ between monoculture or polyculture (Millett *et al.*, 2012), however in polyculture leaves of *F. sylvatica* and *B. pendula* were less enriched in ¹⁵N compared to the leaves of these species growing in monoculture. This difference suggests an incorporation of N fixed by the symbionts of *A. glutinosa*. In other investigations, the contribution of transferred N to total N was 5–15% (Arnebrant *et al.*, 1993) and 1–3% (Ekblad & HussDanell, 1995) on average between *A. glutinosa* and *P. contorta* and *A. incana* and *P. sylvestris*, respectively. Furthermore, leaves of both *F. sylvatica* and *B. pendula* with greater numbers of *A. glutinosa* as direct neighbours were significantly depleted in ¹⁵N compared to leaves of those with fewer *A. glutinosa* as direct neighbours (Millett *et al.*, 2012), suggesting a competition for N as a possible mechanism for exclusion of some of the *B. pendula*. 
In response to elevated CO$_2$, aboveground woody biomass for all 3 species combined was increased by 22% in monocultures. A response of this magnitude is consistent with previously reported woody plant response of 28% calculated from meta-analyses of elevated CO$_2$ experiments (Curtis & Wang, 1998; Ainsworth & Long, 2005) or 23% from four forest FACE experiments after six years of enrichment (Norby et al., 2005). Utilising observations spanning somewhat longer exposure to elevated CO$_2$ (up to 11 years), Norby et al., (2010) have shown that NPP responsiveness decreases in time. The limitation of NPP stimulation may largely be attributed to progressive nitrogen limitation (PNL), however the observed reduction in NPP stimulation was almost entirely accounted for by changes in fine root production. Given the life history character traits of the species chosen in our experimental plantation, it is possible that the increased accretion of woody biomass we observed in polyculture may not decrease as the forest stand develops. The presence of A. glutinosa in the mixture should compensate for increased N uptake and thus negate or at least delay the onset of PNL. Several studies have shown that the rate of N-fixation in the nodules of trees supporting this type of symbiosis increases under elevated CO$_2$, presumably as a result of increased C availability (Hungate et al., 1999; Schortemeyer et al., 2002). B. pendula and F. sylvatica growing in our plantation have been shown to utilize N fixed by A.glutinosa, suggesting that the presence of an N-fixing species might alleviate N limitation for all species grown in a polyculture.

There were considerable temporal differences in the response to elevated CO$_2$ at our site. In the first growing season before canopy closure, all species responded to elevated CO$_2$ enrichment by increasing total biomass by 27-29%. Stimulation of B. pendula began to decline during the second growing season, whereas the response of F. sylvatica declined during the last two growing seasons – an effect often attributed
to acclimation to elevated CO$_2$ (Ainsworth & Long, 2005) or to nutrient limitation (Oren et al., 2001). In the present study leaf N was unaffected by elevated CO$_2$ during all stages of development, and thus it is unlikely that the decreasing overall elevated CO$_2$ effect is due to N limitation. Due to the history of land use at the site, we did not expect lack of N to limit plant growth within the first four years. In fact, foliar N increased while leaf NUE decreased with time in all treatments, indicating sufficient N uptake. In all species pooled together, leaf NUE was increased by elevated CO$_2$ and also by growing trees in a mixture. However, we did not observe any differences in leaf NUE in individual species, suggesting that a different mechanism may explain observed species-specific responses.

Since we observed an expanding system with at least two canopy levels, the developmental phase of the stand and the strength of competition in our experiment must also be considered. Each species used in this study differs in their shade tolerance. Ellenberg (1991) characterised *F. sylvatica*, *A. glutinosa* and *B. pendula* respectively as shade tolerant (3, out of 9), intermediate (5) and light demanding (7). Low leaf mass per leaf unit area and high rate of carbon assimilation per unit leaf area of light demanding species allow rapid occupancy of available space and some canopy light penetration (Niinemets, 2006). Considering only monocultures in 2005, the saplings of each species were initially not influenced by intra-specific competition for light and space, allowing a greater response to elevated CO$_2$. The subsequent decline in response of *F. sylvatica* to elevated CO$_2$, may be explained by strong intraspecific competition through leaf morphology and crown architecture that minimises canopy light penetration. In contrast, *A. glutinosa* sustained the stimulation by elevated CO$_2$, ranging between 25-32% throughout the four year experiment. Claessens et al., (2010) described *A. glutinosa* as fast growing when juvenile, but as a
poor competitor that does not produce shade leaves. Respirational losses of crown shaded leaves may result in a leaf carbon balance that approaches zero which can lead to rapid leaf death (Reich et al., 2009). In our ecosystem, fast juvenile growth coupled with rapid self-pruning enabled A. glutinosa grown in monoculture to fully utilise elevated levels of atmospheric CO$_2$ to accumulate aboveground woody biomass, however, aboveground growth response to elevated CO$_2$ was dramatically reduced when species were grown in polyculture. Initial increases in biomass of F. sylvatica were marginal, eventually becoming suppressed in the last growing seasons. The lack of stimulation of F. sylvatica is most likely due to faster canopy occupation by A. glutinosa and B. pendula under elevated CO$_2$. Changes in leaf area index (LAI) may influence canopy light penetration and inter-specific competition under elevated CO$_2$. In our study, in monocultures the LAI was unaffected by elevated CO$_2$, but was there was a consistently higher trend in B. pendula for the first three years. During the summer of 2006, a severe drought resulted in partial canopy defoliation, which may explain the dramatic LAI increase in 2007. Both species possess indeterminate growth characteristics that enabled an additional leaf flush when environmental conditions improved later in the 2006 season. We propose two mechanisms to explain this phenomena; (i) differences in rooting depth between the two species and (ii) the ability to recover from defoliation related to N storage. A. glutinosa has been characterised as possessing extensive root systems, with particularly deep tap roots that enable it to access water below the normal water table (Schmidt-Vogt, 1971; Claessens et al., 2010). This confers a considerable advantage in leaf production during, and following, drought conditions. The second explanation centres on the storage of N in tree perennial organs which can be re-mobilised and support leaf regrowth after defoliation. In combination with a flush of carbon and organic niorogen
compounds released for root uptake as the abscised litter decomposed mid-growing season, this mechanism may have facilitated the development of leaf primordia and a greater LAI during the following season (Tromp, 1983). Oksanen et al. (2001) found that elevated CO$_2$ consistently increased leaf area index throughout the growing season in aspen, birch and maple stands, which was attributed to larger leaves. In contrast, Gielen et al. (2001) found that leaf area index of $P.$ nigra increased by 225% during the first growing season. However, a post-canopy closure analysis using a fish-eye canopy analyser revealed no increase in leaf area index, which is in agreement with data obtained at the Oak Ridge deciduous closed canopy elevated CO$_2$ experiment (Norby et al., 2003). Our results clearly show that the aboveground response to elevated CO$_2$ is species dependent, but also affected by intra- and inter-specific competition. Indeed, old growth $F.$ sylvatica have been reported to show only a limited response to CO$_2$ enrichment (Körner et al., 2005). In our study, a small, but statistically non-significant positive effect of elevated CO$_2$ on $F.$ sylvatica in polyculture was shown in 2006, a year in which a severe summer drought in June and July resulted in strong leaf loss in $A.$ glutinosa and $B.$ pendula. During this period only 44 mm of precipitation fell, compared to 101, 216 and 85 mm in the same period of 2005, 2007 and 2008 respectively. In July 2006 maximum temperature was 34.5 °C, 10 °C warmer than in other years. The increase in light penetration to the understory formed by $F.$ sylvatica, in combination with improved water use efficiency, may have stimulated a response to elevated CO$_2$, at least until $A.$ glutinosa and $B.$ pendula regrew some of their foliage in late August. The literature suggests that much of the response of trees to elevated CO$_2$ is linked to greater water availability, and that trees may be more drought tolerant under elevated CO$_2$ (Eamus, 1991; Holtum & Winter, 2010;
Leuzinger et al., 2011). If elevated CO$_2$ had conferred a greater tolerance to drought in our experiment we would have expected the highest response to elevated CO$_2$ in 2006, this was clearly not the case for A. glutinosa and B. pendula, however, the severity of the drought in combination with higher temperatures and photosynthetic oxidative stress should also be considered.

To date, the majority of tree elevated CO$_2$ experiments have used monospecific tree stands and report a mean stimulation of NPP for the duration of the observation (Norby et al., 2010). We show that in a short-term empirical study of juvenile deciduous temperate trees grown in polyculture that the aboveground woody biomass response to elevated CO$_2$ was strongly decreased. This result may have implications for estimating global forest response to elevated CO$_2$, as in natural mixed species forest the response to CO$_2$ may be lower than previous estimates. However, caution must be exercised when extrapolating data from small scale temperate plantations, particularly when there is potential for experimental artefacts, arising from CO$_2$ enrichment systems and edge effects influencing the response of saplings planted in complex arrangements at high planting densities. Although providing useful data experimental plantations do not directly mimic the natural species diverse, multi-aged, and complex structures of the majority of the world’s forests that grow in differing biomes, constrained by other physical and environmental drivers. Leuzinger et al. (2011) suggest that an increase in the number of driver variables such as elevated CO$_2$, drought, N addition will dampen ecosystem response to single factors through contrasting driver interactions. Similarly, Langley & Megonigal (2010) could show that in a grassland system, addition of N under high CO$_2$ promoted a shift in community composition to C$_4$ species that were less responsive to CO$_2$, thus decreasing overall community response. Further, Langley & Megonigal (2010)
suggest that if the addition of N favours species that respond strongly to CO$_2$, the
community response to CO$_2$ should increase. In our experimental mixture,
complementary resource acquisition has lead to greater community productivity
which has dampened the aboveground woody biomass response to elevated CO$_2$ even
though the most responsive species in monoculture (A. glutinosa and B. pendula) have
been promoted within the mixed community. This is most likely due to changes in
source-sink relationships and carbon allocation to belowground organs. Indeed, tree
root systems under elevated atmospheric CO$_2$ have been shown to expand deeper into
the soil (Lukac et al., 2003; Iversen, 2010; Smith et al., 2012). Clearly, we are only
beginning to understand how changes in elevated CO$_2$ influenced above- and
belowground processes may alter plant community dynamics.

In conclusion, atmospheric CO$_2$ enrichment did not alter species specific allometric
relationships. Estimation of aboveground biomass stocks and productivity revealed a
differential response to elevated atmospheric CO$_2$. Aboveground biomass responses to
CO$_2$ enrichment were species specific and strongly reduced when species were grown
in polyculture. In monoculture, A. glutinosa produced the largest and most consistent
response, maintaining growth response until the experiment’s conclusion. In contrast,
the growth response of B. pendula and F. sylvatica diminished with time. In
polyculture growth of F. sylvatica was not enhanced by elevated CO$_2$. Our results
suggest that determining how the aboveground biomass response of deciduous species
grown in polyculture differs over single species plantations is imperative to improving
our understanding of future CO$_2$ will impact natural forest community dynamics.

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**Supporting Information**

**Supporting Information Fig. S1** – Effect of CO$_2$ fumigation on diameter at base (A) and height (B) of all trees grown within experimental plots.

**Supporting Information Fig. S2** – Diameter class distributions at the conclusion of the Bangor FACE experiment of individual species grown in monoculture and a three species polyculture under ambient and elevated CO$_2$.

**Supporting Information Fig. S3** – Foliar nitrogen content (a), aboveground NPP (b), and leaf NUE (c) in *A.glutinosa*, *B.pendula* and *F.sylvatica*. 
Table 1 Overall effect of elevated CO$_2$ and probability of significance at the end of 2008 growing season after four years fumigation. The effect of elevated CO$_2$ is expressed as a percentage relative to control plot measurements of tree diameter at 22.5 cm and height of A. glutinosa, B. pendula, F. sylvatica. Trees were grown in monocultures and a three species polyculture. Statistically significant results are emboldened and denoted by an asterisk (**P<0.01).

<table>
<thead>
<tr>
<th>Planting pattern</th>
<th>Species</th>
<th>Diameter Effect</th>
<th>Probability</th>
<th>Height Effect</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono</td>
<td>A. glutinosa</td>
<td>14%</td>
<td><strong>0.007</strong></td>
<td>3%</td>
<td>0.706</td>
</tr>
<tr>
<td></td>
<td>B. pendula</td>
<td>6%</td>
<td>0.146</td>
<td>0%</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td>F. sylvatica</td>
<td>6%</td>
<td>0.603</td>
<td>0%</td>
<td>0.965</td>
</tr>
<tr>
<td>Poly</td>
<td>A. glutinosa</td>
<td>4%</td>
<td>0.618</td>
<td>1%</td>
<td>0.837</td>
</tr>
<tr>
<td></td>
<td>B. pendula</td>
<td>5%</td>
<td>0.614</td>
<td>3%</td>
<td>0.728</td>
</tr>
<tr>
<td></td>
<td>F. sylvatica</td>
<td>-5%</td>
<td>0.483</td>
<td>-12%</td>
<td>0.333</td>
</tr>
</tbody>
</table>
Table 2 Allometric relationship power function scaling coefficients for the three species utilised in this study determined by regression analysis.

<table>
<thead>
<tr>
<th>Species</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alnus glutinosa</em></td>
<td>0.5200</td>
<td>2.020</td>
<td>0.85</td>
</tr>
<tr>
<td><em>Betula pendula</em></td>
<td>0.4414</td>
<td>2.163</td>
<td>0.86</td>
</tr>
<tr>
<td><em>Fagus sylvatica</em></td>
<td>0.6885</td>
<td>1.853</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Table 3 Effect of CO₂ enrichment on aboveground woody biomass of *Alnus glutinosa*, *Betula pendula* and *Fagus sylvatica* when grown in monoculture and in a three species polyculture. Statistically significant results are emboldened and denoted by an asterisk (*P*<0.05).

<table>
<thead>
<tr>
<th>Planting</th>
<th>Species</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mono</strong></td>
<td><em>A. glutinosa</em></td>
<td><em>+29%</em></td>
<td>+25%</td>
<td><em>+28%</em></td>
<td><em>+32%</em></td>
<td>+29%</td>
</tr>
<tr>
<td></td>
<td><em>B. pendula</em></td>
<td>+27%</td>
<td>+13%</td>
<td>+14%</td>
<td>+9%</td>
<td>+16%</td>
</tr>
<tr>
<td></td>
<td><em>F. sylvatica</em></td>
<td>+28%</td>
<td>+33%</td>
<td>+20%</td>
<td>+9%</td>
<td>+22%</td>
</tr>
<tr>
<td><strong>Poly</strong></td>
<td><em>A. glutinosa</em></td>
<td>+13%</td>
<td>+12%</td>
<td>+3%</td>
<td>+8%</td>
<td>+10%</td>
</tr>
<tr>
<td></td>
<td><em>B. pendula</em></td>
<td>+4%</td>
<td>+8%</td>
<td>+6%</td>
<td>+7%</td>
<td>+6%</td>
</tr>
<tr>
<td></td>
<td><em>F. sylvatica</em></td>
<td>+2%</td>
<td>+5%</td>
<td>+2%</td>
<td>-8%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Table 4 F-values and probability of significance for sampling year and sampling year × CO$_2$ treatment interactions from a repeated measures ANOVA of tree diameter, height and aboveground woody biomass for *A. glutinosa*, *B. pendula* and *F. sylvatica* grown in both monoculture and polyculture. Statistically significant results are emboldened and denoted by an asterisk (*$P$<$0.1$, **$P$<$0.05$, ***$P$<$0.001$).

<table>
<thead>
<tr>
<th>Planting Pattern</th>
<th>Species</th>
<th>Source of Variation</th>
<th>Diameter F</th>
<th>Probability</th>
<th>Height F</th>
<th>Probability</th>
<th>Biomass F</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Mono</strong></td>
<td><em>A. glutinosa</em></td>
<td>treatment</td>
<td>7.216</td>
<td>0.036**</td>
<td>0.681</td>
<td>0.441</td>
<td>3.920</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year</td>
<td>506.525</td>
<td><em>&lt;0.001</em>**</td>
<td>512.615</td>
<td><em>&lt;0.001</em>**</td>
<td>253.786</td>
<td><em>&lt;0.001</em>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year×treatment</td>
<td>2.689</td>
<td>0.055</td>
<td>0.603</td>
<td>0.664</td>
<td>5.546</td>
<td>0.008**</td>
</tr>
<tr>
<td></td>
<td><strong>B. pendula</strong></td>
<td>treatment</td>
<td>1.808</td>
<td>0.227</td>
<td>0.076</td>
<td>0.792</td>
<td>1.064</td>
<td>0.342</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year</td>
<td>428.974</td>
<td><em>&lt;0.001</em>**</td>
<td>394.712</td>
<td><em>&lt;0.001</em>**</td>
<td>113.580</td>
<td><em>&lt;0.001</em>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year×treatment</td>
<td>0.610</td>
<td>0.659</td>
<td>0.193</td>
<td>0.940</td>
<td>0.078</td>
<td>0.971</td>
</tr>
<tr>
<td></td>
<td><strong>F. sylvatica</strong></td>
<td>treatment</td>
<td>1.017</td>
<td>0.352</td>
<td>0.576</td>
<td>0.477</td>
<td>0.445</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year</td>
<td>123.828</td>
<td><em>&lt;0.001</em>**</td>
<td>200.403</td>
<td><em>&lt;0.001</em>**</td>
<td>47.454</td>
<td><em>&lt;0.001</em>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year×treatment</td>
<td>0.454</td>
<td>0.769</td>
<td>1.124</td>
<td>0.368</td>
<td>0.250</td>
<td>0.860</td>
</tr>
<tr>
<td></td>
<td><strong>Poly</strong></td>
<td><em>A. glutinosa</em></td>
<td>treatment</td>
<td>0.319</td>
<td>0.592</td>
<td>0.110</td>
<td>0.751</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year</td>
<td>377.886</td>
<td><em>&lt;0.001</em>**</td>
<td>934.984</td>
<td><em>&lt;0.001</em>**</td>
<td>125.788</td>
<td><em>&lt;0.001</em>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year×treatment</td>
<td>0.818</td>
<td>0.526</td>
<td>0.223</td>
<td>0.923</td>
<td>0.179</td>
<td>0.909</td>
</tr>
<tr>
<td></td>
<td><strong>B. pendula</strong></td>
<td>treatment</td>
<td>0.440</td>
<td>0.532</td>
<td>0.368</td>
<td>0.566</td>
<td>0.355</td>
<td>0.573</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year</td>
<td>223.473</td>
<td><em>&lt;0.001</em>**</td>
<td>351.368</td>
<td><em>&lt;0.001</em>**</td>
<td>64.346</td>
<td><em>&lt;0.001</em>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year×treatment</td>
<td>0.245</td>
<td>0.910</td>
<td>0.088</td>
<td>0.985</td>
<td>0.083</td>
<td>0.969</td>
</tr>
<tr>
<td></td>
<td><strong>F. sylvatica</strong></td>
<td>treatment</td>
<td>0.003</td>
<td>0.958</td>
<td>0.695</td>
<td>0.436</td>
<td>0.270</td>
<td>0.622</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year</td>
<td>205.838</td>
<td><em>&lt;0.001</em>**</td>
<td>116.937</td>
<td><em>&lt;0.001</em>**</td>
<td>101.798</td>
<td><em>&lt;0.001</em>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>year×treatment</td>
<td>0.651</td>
<td>0.632</td>
<td>0.950</td>
<td>0.453</td>
<td>1.240</td>
<td>0.325</td>
</tr>
</tbody>
</table>
Table 5 Effect of CO$_2$ enrichment on annual production of aboveground woody biomass in *Alnus glutinosa*, *Betula pendula* and *Fagus sylvatica* when grown in monocultures and polyculture with other species. Statistically significant results are emboldened and denoted by an asterisk (*P*<0.05).

<table>
<thead>
<tr>
<th>Planting</th>
<th>Species</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mono</strong></td>
<td><em>A. glutinosa</em></td>
<td>35%</td>
<td>20%</td>
<td><em>33%</em></td>
<td><em>59%</em></td>
<td>37%</td>
</tr>
<tr>
<td></td>
<td><em>B. pendula</em></td>
<td>32%</td>
<td>-7%</td>
<td>15%</td>
<td>-8%</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td><em>F. sylvatica</em></td>
<td>30%</td>
<td>38%</td>
<td>-4%</td>
<td>-31%</td>
<td>9%</td>
</tr>
<tr>
<td><strong>Poly</strong></td>
<td><em>A. glutinosa</em></td>
<td>27%</td>
<td>13%</td>
<td>-13%</td>
<td>29%</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td><em>B. pendula</em></td>
<td>6%</td>
<td>13%</td>
<td>4%</td>
<td>7%</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td><em>F. sylvatica</em></td>
<td>-2%</td>
<td>9%</td>
<td>-20%</td>
<td>-38%</td>
<td>-13%</td>
</tr>
</tbody>
</table>
Table 6 Leaf nitrogen content (% ± SEM) of *Alnus glutinosa*, *Betula pendula* and *Fagus sylvatica* grown under ambient and elevated CO$_2$. Figures in bold denote CO$_2$ effect significant at $P<0.05$. Source $^a$Ahmed (2006), $^b$Anthony (2007), $^c$Millett *et al.* (2011).

<table>
<thead>
<tr>
<th>Species</th>
<th>2006$^a$</th>
<th>2007$^b$</th>
<th>2008$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient</td>
<td>FACE</td>
<td>Ambient</td>
</tr>
<tr>
<td><em>A. glutinosa</em></td>
<td>4.1 ± 0.5</td>
<td>3.1 ± 0.2</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td><em>B. pendula</em></td>
<td>3.0 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td><em>F. sylvatica</em></td>
<td>2.0 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td><strong>1.6 ± 0.5</strong></td>
</tr>
</tbody>
</table>
Table 7 Analysis of the LAI of trees grown in monoculture and a three species polyculture under ambient and elevated CO$_2$ between 2005-2008 using repeated measures ANOVA. Statistically significant results are emboldened and denoted by an asterisk (\*\(P<0.05\), \***P<0.001\)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Monoculture</th>
<th>Polyculture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-Value</td>
<td>Probability</td>
</tr>
<tr>
<td>year</td>
<td>44.478</td>
<td>(&lt;0.001^{***})</td>
</tr>
<tr>
<td>year \times treatment</td>
<td>1.318</td>
<td>0.283</td>
</tr>
<tr>
<td>year \times species</td>
<td>3.715</td>
<td>(0.020^{*})</td>
</tr>
<tr>
<td>year \times treatment \times species</td>
<td>0.423</td>
<td>0.737</td>
</tr>
</tbody>
</table>
Fig. 1 Layout of ambient and elevated CO$_2$ plots; a = *Alnus glutinosa*, b = *Betula pendula*, F = *Fagus sylvatica*. Each plot contains 27 trees per species. Monoculture species area is indicated by a solid lined oval and three species polyculture plots a dot-dash line oval.
Fig. 2 Mean ± SE aboveground woody biomass for the species grown in monoculture sub-plots under elevated and ambient CO₂ for four years. Aboveground woody biomass was calculated from allometric relationship determined from whole tree harvesting in 2006. Hollow circles indicated elevated atmospheric CO₂ and filled circles indicate ambient CO₂.
Fig. 3 Overyielding (a) and CO₂ fertilisation (b) effects in pooled data for *A. glutinosa*, *B. pendula* and *F. sylvatica*. Overyielding was calculated as aboveground woody biomass measured in polyculture over that predicted from monocultures. Predicted biomass was calculated by taking 1/3 of biomass observed in each species when grown in monoculture. CO₂ fertilisation was calculated as biomass in elevated over ambient CO₂ treatments. Values are mean ± SE, n=4.
Fig. 4 Measured leaf area index for *A. glutinosa* and *B. pendula* grown under ambient and elevated CO$_2$ in monoculture (upper panel) and polyculture (lower panel). Values are mean ± SE.
Fig. 5 Leaf Nitrogen Use Efficiency (NUE) defined as aboveground net primary production per unit of leaf N content. Leaf N Data for (a) *A.glutinosa*, (b) *B.pendula* and (c) *F.sylvatica* are from Ahmed (2006), Anthony (2007) and Millett et al. (2011). Values are mean ± SE, n=4.
**Fig. 6** Leaf Nitrogen Use Efficiency (NUE), defined as aboveground net primary production per unit of leaf N content, in trees grown in monocultures and a three species mixture. Leaf N Data for (a) *A. glutinosa*, (b) *B. pendula* and (c) *F. sylvatica* are from Ahmed (2006), Anthony (2007) and Millett *et al.* (2011). Values are mean ± SE, n=4.