

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

Article

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

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24 Main Body:	7016 words
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26 Materials and Methods:	1635 words
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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 $\mu\text{mol mol}^{-1}$) 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 $12.9 \pm 1.4 \text{ kg m}^{-2}$ to $18.9 \pm 1.0 \text{ kg m}^{-2}$, whereas in an elevated CO₂ atmosphere aboveground woody biomass increased from $15.2 \pm 0.6 \text{ kg m}^{-2}$ to $20.2 \pm 0.6 \text{ kg m}^{-2}$. 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*), biomass, allometry, polyculture, monoculture, overyielding.

59 **Introduction**

60 Forests occupy one third of the land surface of the Earth, and account for almost half
61 of carbon stored in the terrestrial biosphere (Schlesinger & Lichter, 2001). In a
62 summary of studies conducted to investigate the effects of increased atmospheric CO₂
63 on forest C cycles, Norby *et al.*, (2005) calculated that an enrichment of 200 ppm CO₂
64 above the current ambient CO₂ level caused a 23% median increase of forest net
65 primary productivity. However, interactions with other environmental factors may
66 dampen such response at larger temporal or spatial scales (Leuzinger *et al.*, 2011).
67 Nevertheless, increasing atmospheric CO₂ concentrations may fundamentally alter
68 forest ecosystem functioning by altering species growth, resource use and community
69 interactions (Eamus & Jarvis, 1989). As forests are inextricably linked to the global
70 carbon cycle, elevated CO₂ driven environmental change may impact upon global
71 carbon storage in phytomass, complex biogeochemical feedback mechanisms and
72 ultimately long term C sequestration in soils.

73 Empirical studies on woody plants exposed to elevated atmospheric CO₂ have
74 demonstrated that growth and aboveground biomass production in woody plants
75 increases, but that there is a considerable variation in response (Curtis & Wang,
76 1998). The observed variation of responses to elevated CO₂ has been attributed to a
77 large number of confounding factors, such as the length of study, interactions with
78 other environmental stresses, plant functional group, species morphological
79 physiology (Poorter, 1993), symbiotic associations (Godbold *et al.*, 1997) and
80 community dynamics (Kozovits *et al.*, 2005). Recent research efforts have been
81 focused on studying whole ecosystem responses in near-natural conditions chiefly
82 achieved by employing Free Air Carbon dioxide Enrichment (FACE) facilities
83 (Hattenschwiler *et al.*, 2002; Karnosky *et al.*, 2003; Körner *et al.*, 2005; Hoosbeek *et*

84 *al.*, 2011). Körner (2006) has suggested dividing elevated CO₂ studies into the
85 following two types: (i) high abundance of major resources other than carbon –
86 ‘decoupled’ systems and (ii) near to steady-state nutrient cycling and full canopy
87 development – ‘coupled systems’. Type I systems include the present study, aspen
88 FACTS II FACE (Karnosky *et al.*, 2003), and EuroFACE (Calfapietra *et al.*, 2003)
89 experiments. The remaining three (type II) experiments have used CO₂ enrichment in
90 stands with an already closed canopy. The Oak Ridge (Norby *et al.*, 2002) and
91 DukeFACE (Oren *et al.*, 2001) experiments both started enrichment *ca.* 10-20 years
92 after planting, while at the Basel Web-FACE (Körner *et al.*, 2005) enrichment was
93 conducted in a mature deciduous forest comprised of four species more than 100 years
94 old. Using data from four of these studies (DukeFACE, FACTS II FACE, Oak Ridge
95 and EuroFACE), Norby *et al.*, (2005) demonstrated that an enrichment of 200 ppm
96 CO₂ above the current ambient CO₂ level caused a 23% median increase of forest net
97 primary productivity. This conclusion was largely based on the initial response of
98 forest ecosystems to elevated CO₂. Subsequent investigations have shown that this
99 response may not be maintained over a longer time horizon (Norby *et al.*, 2010), as
100 the response to elevated CO₂ has been found to both decline (Norby *et al.*, 2010) or be
101 maintained (Drake *et al.*, 2011; Zak *et al.*, 2011) after 10-11 years of exposure. In
102 both of these examples, the response to elevated CO₂ was likely mediated by N
103 availability. The decline in response to elevated CO₂ was attributed to N limitation
104 (Norby *et al.*, 2010), while no change in response was a result of greater N cycling
105 (Zak *et al.*, 2011). Comparison of these two studies clearly demonstrates that nutrient
106 availability, in particular N, is a strong factor mediating the response of woody plants
107 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 CO₂ 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 CO₂ 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 CO₂.

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 mediteranean 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 CO₂ 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 CO₂ (Bazzaz & Miao, 1993), the influence of elevated CO₂ on tree competition, and the influence of tree biodiversity on community response to CO₂ has not been investigated.

The objectives of this work were to investigate the effects of elevated CO₂ (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 CO₂ over four years. We tested the hypothesis that interspecific competition modifies the response of tree species to elevated CO₂.

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 CO₂ 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 (Aguilar *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 CO₂ 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 CO₂ enrichment system in the treatment plots. Ambient CO₂ control plots were identical to the treatment plots, but for the absence of CO₂ 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 CO₂ injection (Okada *et al.*, 2001). In the first two growing seasons, CO₂ 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 CO₂ delivery was achieved using equipment and software modified from EuroFACE (Miglietta *et al.*, 2001). The target concentration in the elevated CO₂ plots was ambient plus 200 ppm. The elevated CO₂ concentrations, measured at 1 minute intervals, were within 30% deviation from the pre-set target concentration of 580 ppm CO₂ for 75-79% of the time during the photosynthetically active (daylight hours between budburst until leaf abscission) period of 2005 – 2008. Vertical profiles of CO₂ 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 CO₂ fumigation on diameter and height of trees grown within the plots was not modified by the distance from the CO₂ delivery pipe (Supporting Information

Fig. S1). The CO₂ used for enrichment originated from natural gas and had a $\delta^{13}\text{C}$ 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 CO₂ 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).

Eqn 1

Eqn 2

Stem volume index (basal diameter² × 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).

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

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 °C 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 °C 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 pseudoreplication 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 CO₂, 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 CO₂ in any of the species. Tree height was unaffected by elevated CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ treatment more than those grown in the three species polyculture. Fig. 3 and Table 3 detail the

relationship between time and biomass accrue-
ment for all species in ambient and
elevated atmospheric CO₂. Under ambient CO₂ both *A. glutinosa* and *B. pendula*
accumulated aboveground woody biomass faster in the polyculture than in the
monocultures. The influence of elevated CO₂ 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 × treatment interactions for any
species in the polyculture or for *B. pendula* and *F. sylvatica* in the monocultures.
However, there was a significant year × treatment interaction for *A. glutinosa*
($P=0.008$). Elevated CO₂ 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 CO₂ 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
CO₂, 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
CO₂ 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 CO₂ on overyielding in the mixture ($P=0.094$), nor did we observe any modification of the CO₂ 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 $16.5 \pm 0.8 \text{ kg m}^{-2}$ in ambient CO₂ plots and $19.3 \pm 0.4 \text{ kg m}^{-2}$ in elevated CO₂ plots, a significant increase of 17% ($P=0.022$). The contribution of aboveground woody biomass within the elevated CO₂ plots followed the order *B. pendula* ($10.1 \pm 0.0 \text{ kg m}^{-2}$), *A. glutinosa* ($8.6 \pm 0.6 \text{ kg m}^{-2}$) and *F. sylvatica* ($0.6 \pm 0.0 \text{ kg m}^{-2}$). A significant 16% ($P=0.046$) increase in aboveground woody biomass was observed in *B. pendula* in response to CO₂ treatment. Pooling the values for each species, in the monocultures the aboveground woody biomass was $12.9 \pm 1.4 \text{ kg m}^{-2}$ in ambient, and $15.2 \pm 0.6 \text{ kg m}^{-2}$ in elevated CO₂ treatments. Polyculture aboveground woody biomass reached $18.9 \pm 1.0 \text{ kg m}^{-2}$ in ambient and $20.2 \pm 0.6 \text{ kg m}^{-2}$ in elevated CO₂ treatments. This resulted in an increase in aboveground woody biomass under elevated CO₂ of 18% in monoculture and 7% in polyculture.

To summarise, pooled aboveground woody biomass was significantly affected by elevated CO₂ ($P=0.022$). 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 CO₂ (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 CO₂ 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 CO₂ on leaf NUE in individual species in 2008.

Leaf Area Index

Repeated measures ANOVA showed a significant year $\times$ species interaction for species grown in monoculture ($P<0.05$) and polyculture ($P<0.001$; Table 7). The response of LAI to elevated CO₂ 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 CO₂ enrichment LAI of *B. pendula* was between 1.1-3.2 m² m⁻² in ambient CO₂ and 0.8-4.0 m² m⁻² in elevated CO₂ plots, whereas LAI of *A. glutinosa* was between 1.4-7.6 m² m⁻² and 1.4-8.2 m² m⁻² in ambient and elevated CO₂ plots respectively (Fig. 4). Elevated CO₂ 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*,

478 suggests that some *B. pendula* are being excluded. In our study, we did not
479 systematically determine crown architecture, but observed that in polyculture both *B.*
480 *pendula* and shorter *A. glutinosa* had deeper crowns. Indeed, we saw higher LAI in *A.*
481 *glutinosa* and *B. pendula* in polyculture compared to monocultures, but no difference
482 in stem height, which suggests alteration of crown architecture between monoculture
483 and polyculture grown trees. Claessen *et al*, (2010) suggest that *A. glutinosa* grown in
484 monoculture produces a straight bole and round crown, whereas when grown in
485 admixture with other species forms a stratified canopy. In the meta-analysis of species
486 richness productivity relationships by Zhang *et al.*, (2012), heterogeneity of shade
487 tolerance was the second most important factor explaining increased productivity in
488 mixtures. In addition to an aboveground partitioning of canopy space, an increase in
489 N availability via the N-fixing *A. glutinosa* could also be a factor in the higher
490 productivity of the polyculture. In *A. glutinosa* under ambient CO₂, the amount of N
491 content in the leaves did not differ between monoculture or polyculture (Millett *et al.*,
492 2012), however in polyculture leaves of *F. sylvatica* and *B. pendula* were less
493 enriched in ¹⁵N compared to the leaves of these species growing in monoculture. This
494 difference suggests an incorporation of N fixed by the symbionts of *A. glutinosa*. In
495 other investigations, the contribution of transferred N to total N was 5–15%
496 (Arnebrant *et al.*, 1993) and 1–3% (Ekblad & HussDanell, 1995) on average between
497 *A. glutinosa* and *P. contorta* and *A. incana* and *P. sylvestris*, respectively.
498 Furthermore, leaves of both *F. sylvatica* and *B. pendula* with greater numbers of *A.*
499 *glutinosa* as direct neighbours were significantly depleted in ¹⁵N compared to leaves
500 of those with fewer *A. glutinosa* as direct neighbours (Millett *et al.*, 2012), suggesting
501 a competition for N as a possible mechanism for exclusion of some of the *B. pendula*.

In response to elevated CO₂, 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 CO₂ 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 CO₂ (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 accruelement 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 CO₂, 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 CO₂ at our site. In the first growing season before canopy closure, all species responded to elevated CO₂ 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 CO₂ (Ainsworth & Long, 2005) or to nutrient limitation (Oren *et al.*, 2001). In the present study leaf N was unaffected by elevated CO₂ during all stages of development, and thus it is unlikely that the decreasing overall elevated CO₂ 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 CO₂ 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 CO₂. The subsequent decline in response of *F. sylvatica* to elevated CO₂, 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 CO₂, ranging between 25-32% throughout the four year experiment. Claessens *et al.*, (2010) described *A. glutinosa* as fast growing when juvenile, but as a

552 poor competitor that does not produce shade leaves. Respirational losses of crown
553 shaded leaves may result in a leaf carbon balance that approaches zero which can
554 lead to rapid leaf death (Reich *et al.*, 2009). In our ecosystem, fast juvenile growth
555 coupled with rapid self-pruning enabled *A. glutinosa* grown in monoculture to fully
556 utilise elevated levels of atmospheric CO₂ to accumulate aboveground woody
557 biomass, however, aboveground growth response to elevated CO₂ was dramatically
558 reduced when species were grown in polyculture. Initial increases in biomass of *F.*
559 *sylvatica* were marginal, eventually becoming suppressed in the last growing seasons.
560 The lack of stimulation of *F. sylvatica* is most likely due to faster canopy occupation
561 by *A. glutinosa* and *B. pendula* under elevated CO₂. Changes in leaf area index (LAI)
562 may influence canopy light penetration and inter-specific competition under elevated
563 CO₂. In our study, in monocultures the LAI was unaffected by elevated CO₂, but was
564 there was a consistently higher trend in *B. pendula* for the first three years. During the
565 summer of 2006, a severe drought resulted in partial canopy defoliation, which may
566 explain the dramatic LAI increase in 2007. Both species possess indeterminate growth
567 characteristics that enabled an additional leaf flush when environmental conditions
568 improved later in the 2006 season. We propose two mechanisms to explain this
569 phenomena; (i) differences in rooting depth between the two species and (ii) the
570 ability to recover from defoliation related to N storage. *A. glutinosa* has been
571 characterised as possessing extensive root systems, with particularly deep tap roots
572 that enable it to access water below the normal water table (Schmidt-Vogt, 1971;
573 Claessens *et al.*, 2010). This confers a considerable advantage in leaf production
574 during, and following, drought conditions. The second explanation centres on the
575 storage of N in tree perennial organs which can be re-mobilised and support leaf
576 regrowth after defoliation. In combination with a flush of carbon and organic nitrogen

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 CO₂ 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 CO₂ experiment (Norby *et al.*, 2003.).

Our results clearly show that the aboveground response to elevated CO₂ 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 CO₂ enrichment (Körner *et al.*, 2005). In our study, a small, but statistically non-significant positive effect of elevated CO₂ 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 CO₂, 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 CO₂ is linked to greater water availability, and that trees may be more drought tolerant under elevated CO₂ (Eamus, 1991; Holtum & Winter, 2010;

Leuzinger *et al.*, 2011). If elevated CO₂ had conferred a greater tolerance to drought in our experiment we would have expected the highest response to elevated CO₂ 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 CO₂ 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 CO₂ was strongly decreased. This result may have implications for estimating global forest response to elevated CO₂, as in natural mixed species forest the response to CO₂ 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 CO₂ 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 CO₂, 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 CO₂ promoted a shift in community composition to C₄ species that were less responsive to CO₂, thus decreasing overall community response. Further, Langley & Megonigal (2010)

suggest that if the addition of N favours species that respond strongly to CO₂, the community response to CO₂ 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 CO₂ 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 CO₂ 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 CO₂ influenced above- and belowground processes may alter plant community dynamics.

In conclusion, atmospheric CO₂ enrichment did not alter species specific allometric relationships. Estimation of aboveground biomass stocks and productivity revealed a differential response to elevated atmospheric CO₂. Aboveground biomass responses to CO₂ 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 CO₂. 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 CO₂ will impact natural forest community dynamics.

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Supporting Information

Supporting Information Fig. S1 – Effect of CO₂ 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 CO₂.

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 CO₂ and probability of significance at the end of 2008 growing season after four years fumigation. The effect of elevated CO₂ 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 (** $P<0.01$).

Planting pattern	Species	Diameter		Height	
		Effect	Probability	Effect	Probability
<i>Mono</i>	<i>A. glutinosa</i>	14%	0.007**	3%	0.706
	<i>B. pendula</i>	6%	0.146	0%	0.935
	<i>F. sylvatica</i>	6%	0.603	0%	0.965
<i>Poly</i>	<i>A. glutinosa</i>	4%	0.618	1%	0.837
	<i>B. pendula</i>	5%	0.614	3%	0.728
	<i>F. sylvatica</i>	-5%	0.483	-12%	0.333

Table 2 Allometric relationship power function scaling coefficients for the three species utilised in this study determined by regression analysis.

Species	a	b	R^2
<i>Alnus glutinosa</i>	0.5200	2.020	0.85
<i>Betula pendula</i>	0.4414	2.163	0.86
<i>Fagus sylvatica</i>	0.6885	1.853	0.78

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 (* $P<0.05$).

Planting	Species	2005	2006	2007	2008	Overall
<i>Mono</i>	<i>A. glutinosa</i>	*+29%	+25%	*+28%	*+32%	+29%
	<i>B. pendula</i>	+27%	+13%	+14%	+9%	+16%
	<i>F. sylvatica</i>	+28%	+33%	+20%	+9%	+22%
<i>Poly</i>	<i>A. glutinosa</i>	+13%	+12%	+3%	+8%	+10%
	<i>B. pendula</i>	+4%	+8%	+6%	+7%	+6%
	<i>F. sylvatica</i>	+2%	+5%	+2%	-8%	0%

Table 4 F-values and probability of significance for sampling year and sampling year $\times$ CO₂ 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 (* $P < 0.1$, ** $P < 0.05$, *** $P < 0.001$).

Planting Pattern	Species	Source of Variation	Diameter		Height		Biomass	
			F	Probability	F	Probability	F	Probability
Mono	<i>A. glutinosa</i>	treatment	7.216	0.036 **	0.681	0.441	3.920	0.095 *
		year	506.525	<0.001 ***	512.615	<0.001 ***	253.786	<0.001 ***
		year $\times$ treatment	2.689	0.055	0.603	0.664	5.546	0.008 **
	<i>B. pendula</i>	treatment	1.808	0.227	0.076	0.792	1.064	0.342
		year	428.974	<0.001 ***	394.712	<0.001 ***	113.580	<0.001 ***
		year $\times$ treatment	0.610	0.659	0.193	0.940	0.078	0.971
	<i>F. sylvatica</i>	treatment	1.017	0.352	0.576	0.477	0.445	0.529
		year	123.828	<0.001 ***	200.403	<0.001 ***	47.454	<0.001 ***
		year $\times$ treatment	0.454	0.769	1.124	0.368	0.250	0.860
Poly	<i>A. glutinosa</i>	treatment	0.319	0.592	0.110	0.751	0.271	0.622
		year	377.886	<0.001 ***	934.984	<0.001 ***	125.788	<0.001 ***
		year $\times$ treatment	0.818	0.526	0.223	0.923	0.179	0.909
	<i>B. pendula</i>	treatment	0.440	0.532	0.368	0.566	0.355	0.573
		year	223.473	<0.001 ***	351.368	<0.001 ***	64.346	<0.001 ***
		year $\times$ treatment	0.245	0.910	0.088	0.985	0.083	0.969
	<i>F. sylvatica</i>	treatment	0.003	0.958	0.695	0.436	0.270	0.622
		year	205.838	<0.001 ***	116.937	<0.001 ***	101.798	<0.001 ***
		year $\times$ treatment	0.651	0.632	0.950	0.453	1.240	0.325

Table 5 Effect of CO₂ 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 (* $P<0.05$).

Planting	Species	2005	2006	2007	2008	Overall
<i>Mono</i>	<i>A. glutinosa</i>	35%	20%	*33%	*59%	37%
	<i>B. pendula</i>	32%	-7%	15%	-8%	8%
	<i>F. sylvatica</i>	30%	38%	-4%	-31%	9%
<i>Poly</i>	<i>A. glutinosa</i>	27%	13%	-13%	29%	14%
	<i>B. pendula</i>	6%	13%	4%	7%	8%
	<i>F. sylvatica</i>	-2%	9%	-20%	-38%	-13%

Table 6 Leaf nitrogen content (% $\pm$ SEM) of *Alnus glutinosa*, *Betula pendula* and *Fagus sylvatica* grown under ambient and elevated CO₂. Figures in bold denote CO₂ effect significant at $P < 0.05$. Source ^aAhmed (2006), ^bAnthony (2007), ^cMillett *et al.* (2011).

Species	2006 ^a		2007 ^b		2008 ^c	
	Ambient	FACE	Ambient	FACE	Ambient	FACE
<i>A. glutinosa</i>	4.1 $\pm$ 0.5	3.1 $\pm$ 0.2	3.4 $\pm$ 0.2	3.7 $\pm$ 0.2	4.1 $\pm$ 0.0	3.9 $\pm$ 0.1
<i>B. pendula</i>	3.0 $\pm$ 0.1	2.7 $\pm$ 0.1	2.6 $\pm$ 0.5	2.5 $\pm$ 0.2	3.7 $\pm$ 0.1	3.8 $\pm$ 0.2
<i>F. sylvatica</i>	2.0 $\pm$ 0.1	2.0 $\pm$ 0.1	1.6 $\pm$ 0.5	3.7 $\pm$ 0.1	3.0 $\pm$ 0.1	3.1 $\pm$ 0.1

Table 7 Analysis of the LAI of trees grown in monoculture and a three species polyculture under ambient and elevated CO₂ between 2005-2008 using repeated measures ANOVA. Statistically significant results are emboldened and denoted by an asterisk (* $P < 0.05$, *** $P < 0.001$)

Source of Variation	Monoculture		Polyculture	
	F-Value	Probability	F-Value	Probability
year	44.478	<0.001***	33.451	<0.001***
year × treatment	1.318	0.283	0.106	0.956
year × species	3.715	0.020*	19.008	<0.001***
year × treatment × species	0.423	0.737	1.174	0.333

Fig. 1 Layout of ambient and elevated CO₂ 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.

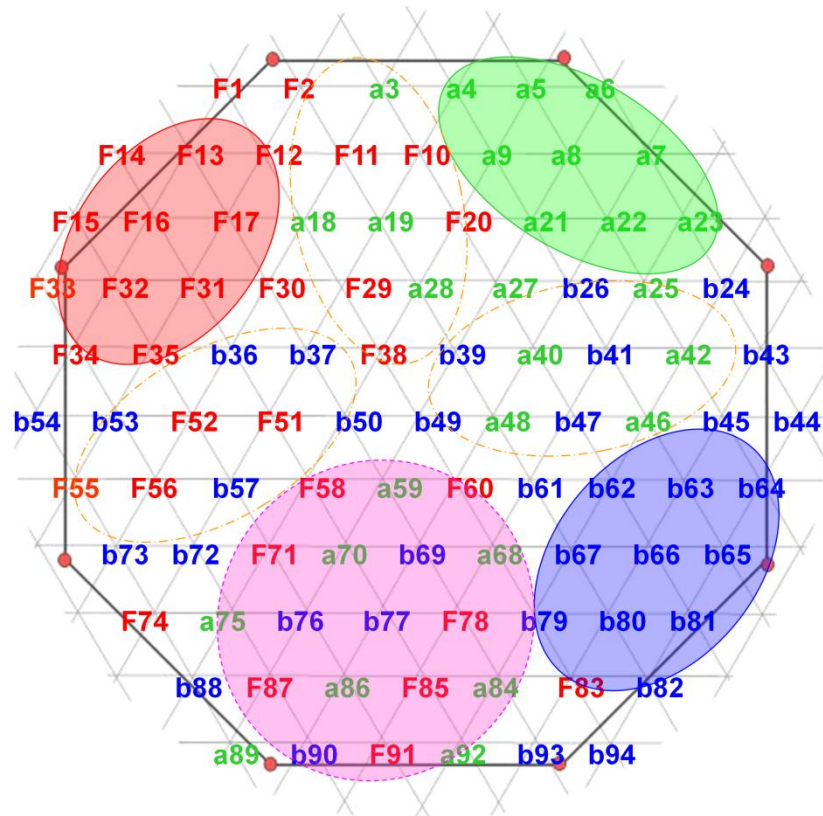


Fig. 2 Mean $\pm$ SE aboveground woody biomass for the species grown in monoculture sub-plots under elevated and ambient CO₂ for four years. Aboveground woody biomass was calculated from allometric relationship determined from whole tree harvesting in 2006. Hollow circles indicated elevated atmospheric CO₂ and filled circles indicate ambient CO₂.

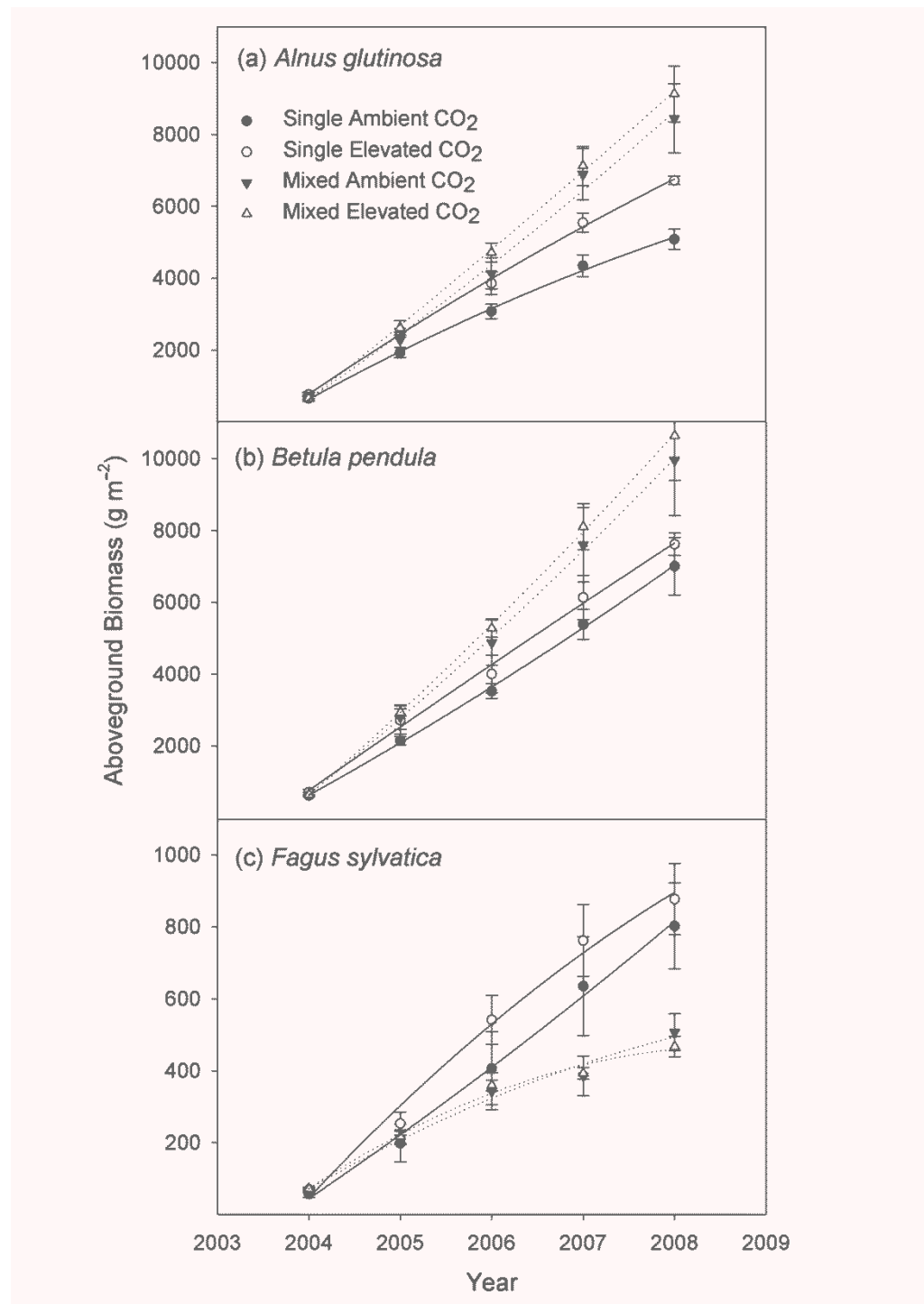


Fig. 3 Overyielding (a) and CO₂ fertilisation (b) effects in pooled data for *A.glutinosa*, *B.pendula* and *F.sylvatica*. Overyielding was calculated as aboveground woody biomass measured in polyculture over that predicted from monocultures. Predicted biomass was calculated by taking 1/3 of biomass observed in each species when grown in monoculture. CO₂ fertilisation was calculated as biomass in elevated over ambient CO₂ treatments. Values are mean $\pm$ SE, $n=4$.

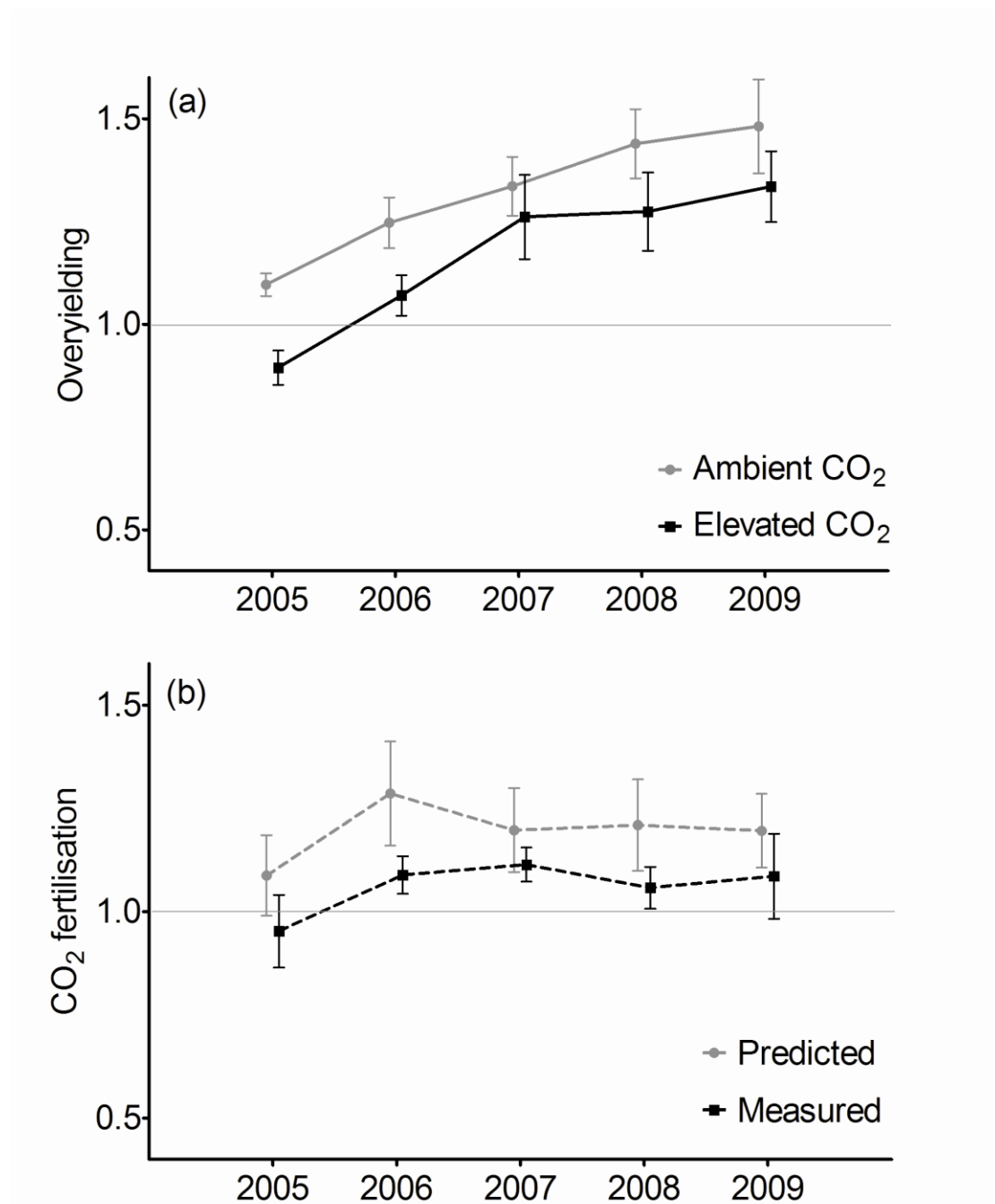


Fig. 4 Measured leaf area index for *A. glutinosa* and *B. pendula* grown under ambient and elevated CO₂ in monoculture (upper panel) and polyculture (lower panel). Values are mean $\pm$ SE.

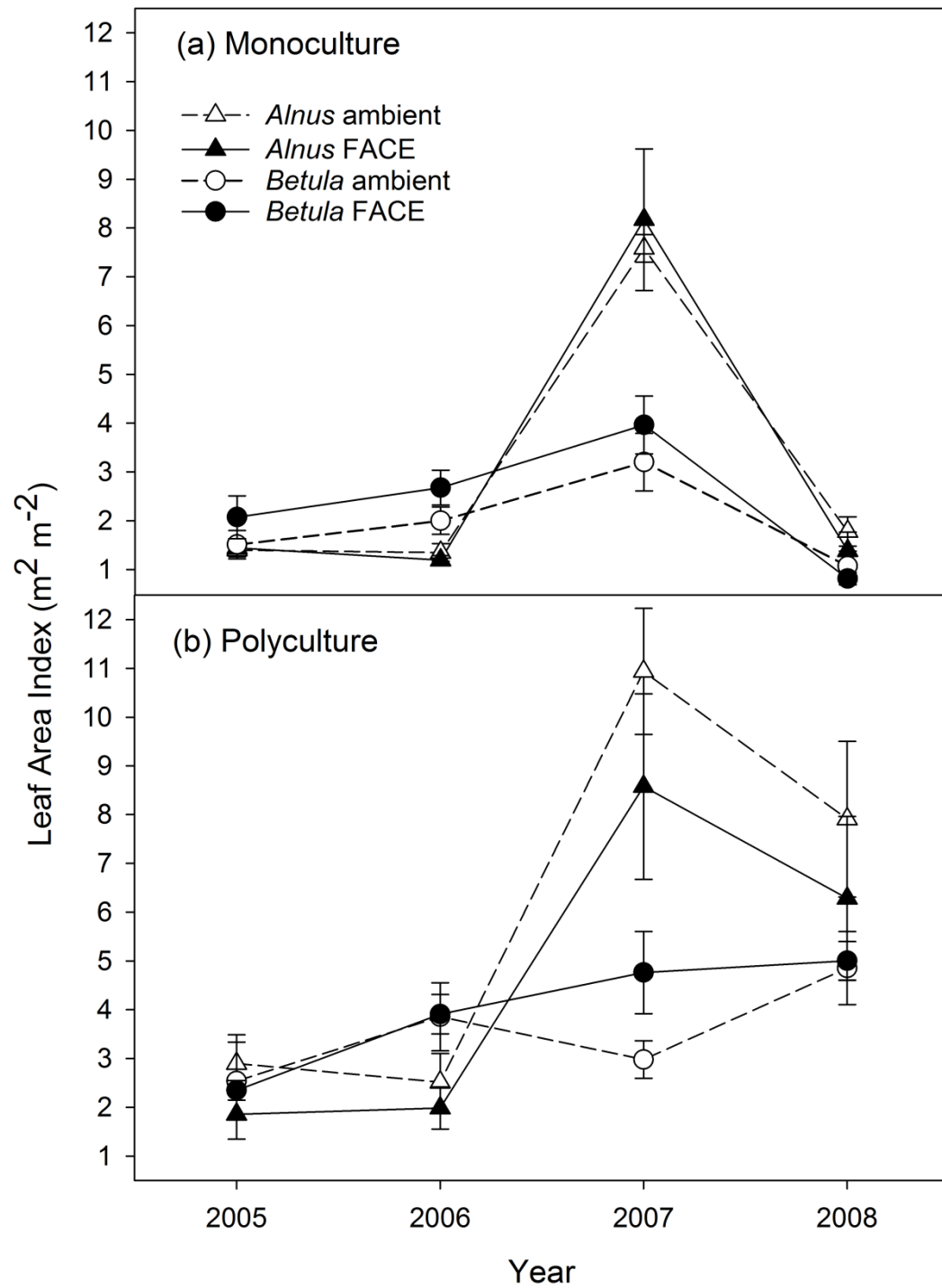


Fig. 5 Leaf Nitrogen Use Efficiency (NUE) defined as aboveground net primary production per unit of leaf N content. Leaf N Data for (a) *A.glutinosa*, (b) *B.pendula* and (c) *F.sylvatica* are from Ahmed (2006), Anthony (2007) and Millett *et al.* (2011). Values are mean $\pm$ SE, $n=4$.

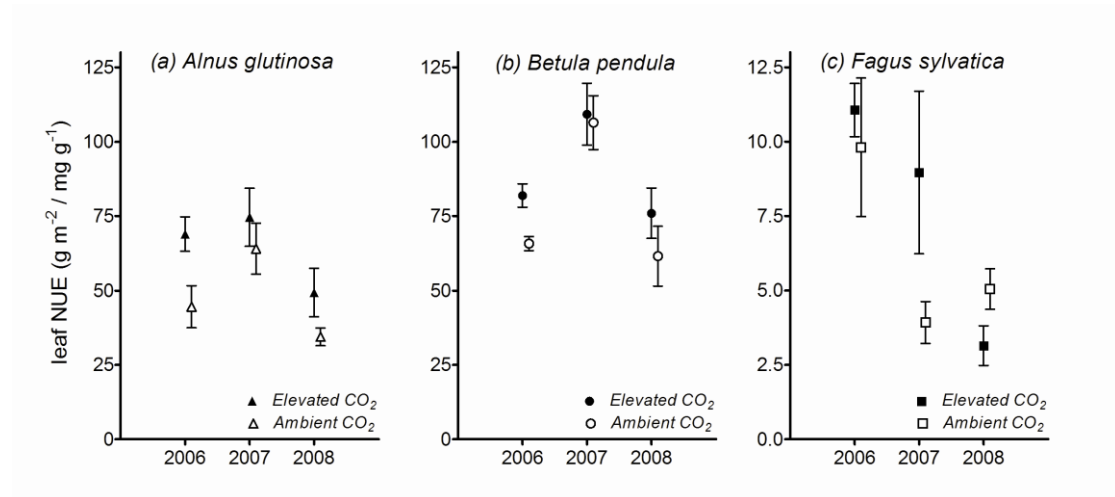


Fig. 6 Leaf Nitrogen Use Efficiency (NUE), defined as aboveground net primary production per unit of leaf N content, in trees grown in monocultures and a three species mixture. Leaf N Data for (a) *A.glutinosa*, (b) *B.pendula* and (c) *F.sylvatica* are from Ahmed (2006), Anthony (2007) and Millett *et al.* (2011). Values are mean $\pm$ SE, $n=4$.

