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

Article

Accepted Version

Smith, A. R., Lukac, M. ORCID: https://orcid.org/0000-0002-8535-6334, Hood, R., Healey, J. R., Miglietta, F. and Godbold, D. L. (2013) Elevated CO2 enrichment induces a differential biomass response in a mixed species temperate forest plantation. New Phytologist, 198 (1). pp. 156-168. ISSN 1469-8137 doi: https://doi.org/10.1111/nph.12136 Available at https://centaur.reading.ac.uk/30144/

To link to this article DOI: http://dx.doi.org/10.1111/nph.12136

Publisher: Wiley-Blackwell

# Elevated $CO_2$ enrichment induces a differential biomass response in a mixed species temperate forest plantation

Andrew R. Smith[1,6], Martin Lukac[2], Robin Hood[1], John R. Healey[1], Franco Miglietta[3,5],Douglas L. Godbold[4]

[1]School of the Environment, Natural Resources and Geography, Bangor University, Gwynedd, LL57 2UW, UK. [2]School of Agriculture, Policy and Development, University of Reading, Reading, Berkshire, RG6 6AR, UK. [3]IBIMET-CNR, Via Caproni, 8-50145 Firenze, Italy. [4]Institute of Forest Ecology, Universität für Bodenkultur (BOKU), 1190 Vienna, Austria. [5]FoxLab, Fondazione E. Mach, Via Mach 1, 38010 San Michele a/Adige (TN), Italy. [6]Centre for Ecology & Hydrology, Environment Centre Wales, Bangor, Gwynedd, LL57 2UW, UK.

Author for correspondence:
Andrew R. Smith
School of the Environment, Natural Resources and Geography,
Environment Centre Wales
Bangor University,
Bangor, Gwynedd, LL57 2UW, UK
Corresponding author Tel.: +44 1248 383052
Corresponding author E-mail: a.r.smith@ceh.ac.uk

| | |
|---|---|
| Main Body: | 7016 words |
| Introduction: | 1347 words |
| Materials and Methods: | 1635 words |
| Results: | 1348 words |
| Discussion: | 2680 words |
| Acknowledgements: | 89 words |
| Number of Figures: | 6 |
| Number of Tables: | 7 |

## Summary

- In a free-air $CO_2$ 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 $CO_2$ (580 µmol $mol^{-1}$) for four years, and aboveground growth characteristics measured.
- In monoculture, the mean effect of $CO_2$ 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ than in an ambient atmosphere (+18%).
- Our results show that the aboveground response to elevated $CO_2$ is significantly affected by intra- and inter-specific competition, and that elevated $CO_2$ response may be reduced in forest communities comprised of tree species with contrasting functional traits.

**keywords:**

Free-air $CO_2$ enrichment (FACE), temperate forest, alder (*Alnus gl$_u$tinosa*) , silver birch (*Betula pendula*) , European beech (*Fagus sylvatica*) , biomass, allometry, polyculture, monoculture, overyielding.

## 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* *al.*, 2011). Körner (2006) has suggested dividing elevated $CO_2$ 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 $CO_2$ 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 $CO_2$ above the current ambient $CO_2$ 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 $CO_2$. 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 $CO_2$ 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 $CO_2$ was likely mediated by N availability. The decline in response to elevated $CO_2$ 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 nutient availability, in particular N,  is a strong factor mediating the response of woody plants to elevated $CO_2$.

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 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 $CO_2$ 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 ºC 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 Palezoic 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 1–2º 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 90º 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 $\delta^{13}C$ 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).

Eqn 1

Eqn 2

Stem volume index (basal diameter$^2$ × 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).

$$B_{mixture} = \left(\frac{1}{3} \times B_{A\ln us}\right) + \left(\frac{1}{3} \times B_{Betula}\right) + \left(\frac{1}{3} \times B_{Fagus}\right) \qquad \text{Eqn 3}$$

*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 $m^2$ 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. 3 and Table 3 detail the relationship between time and biomass accruement 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 × 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 $CO_2$ 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 species in 2008.

### *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 $CO_2$ 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 $CO_2$, 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 $^{15}N$ 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 $^{15}N$ 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 accruement 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 nirogen 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 ºC, 10 ºC 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.

## Acknowledgements

The development of BangorFACE site infrastructure was funded by SRIF. We thank the Aberystwyth and Bangor Universities Partnership Centre for Integrated Research in the Rural Environment and the Forestry Commission Wales for financially supporting the running costs of the experiment. Andrew Smith was supported by the Sir Williams Roberts PhD Scholarship match funded by the Drapers' Company. Many thanks to Michael Bambrick and Gordon Turner for technical assistance throughout the BangorFACE experiment. We thank David Ellsworth and two anonymous reviewers for helpful comments in the revision of this manuscript.

## References

**Aguiar FC, Ferreira MT, Moreira I. 2001.** Exotic and native vegetation establishment following channelization of a western Iberian river. *Regulated Rivers-Research & Management* **17**(4-5): 509-526.

**Ahmed IUMT, Bangor University. 2006.** *Leaf decomposition of birch (Betula pendula), alder (Alnus glutinosa) and beech (Fagus sylvatica) grown under elevated atmospheric $CO_2$.* Bangor University Bangor.

**Ainsworth EA, Long SP. 2005.** What have we learned from 15 years of free-air $CO_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist* **165**(2): 351-371.

**Anthony RVE. 2007.** *The use of near infrared spectroscopy to investigate lignin change under the effects of elevated carbon dioxide for three native broadleaved tree species.* Bangor University Bangor.

**Arnebrant K, Ek H, Finlay RD, Soderstrom B. 1993.** Nitrogen translocation between *Alnus glutinosa* (L) Gaertn seedlings inoculated with *Frankia* Sp and *Pinus contorta* Doug Ex-Loud seedlings connected by a common ectomycorrhizal mycelium. *New Phytologist* **124**(2): 231-242.

**Bartelink HH. 1997.** Allometric relationships for biomass and leaf area of beech (*Fagus sylvatica* L). *Annales Des Sciences Forestieres* **54**(1): 39-50.

**Bazzaz FA, Miao SL. 1993.** Successional status, seed size, and responses of tree seedlings to $CO_2$, light and nutrients. *Ecology* **74**(1): 104-112.

**Braun HJ. 1974.** Rhytmus und grösse von wachstum, wasserverbrauch und produktivität des wasserverbrauches bei holzpflanzen. *Allgemeine Forst- und Jagdzeitung* **145**(5): 81-86.

**Calfapietra C, Gielen B, Galema ANJ, Lukac M, De Angelis P, Moscatelli MC, Ceulemans R, Scarascia-Mugnozza G. 2003.** Free-air $CO_2$ enrichment (FACE) enhances biomass production in a short-rotation poplar plantation. *Tree Physiology* **23**(12): 805-814.

**Claessens H, Oosterbaan A, Savill P, Rondeux J. 2010.** A review of the characteristics of black alder (*Alnus glutinosa* (L.) Gaertn.) and their implications for silvicultural practices. *Forestry* **83**(2): 163-175.

**Curtis PS, Wang XZ. 1998.** A meta-analysis of elevated $CO_2$ effects on woody plant mass, form, and physiology. *Oecologia* **113**(3): 299-313.

**DeClerck FAJ, Barbour MG, Sawyer JO. 2006.** Species richness and stand stability in conifer forests of the Sierra Nevada. *Ecology* **87**(11): 2787-2799.

**Delagrange S, Montpied P, Dreyer E, Messier C, Sinoquet H. 2006.** Does shade improve light interception efficiency? A comparison among seedlings from shade-tolerant and -intolerant temperate deciduous tree species. *New Phytologist* **172**(2): 293-304.

**Drake JE, Gallet-Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB, Johnsen KS, Lichter J, McCarthy HR, McCormack ML, Moore DJP, Oren R, Palmroth S, Phillips RP, Pippen JS, Pritchard SG, Treseder KK, Schlesinger WH, DeLucia EH, Finzi AC. 2011.** Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated $CO_2$. *Ecology Letters* **14**(4): 349-357.

**Eamus D, Jarvis PG. 1989.** The direct effects of increase in the global atmospheric $CO_2$ concentration on natural and commercial temperate trees and forests. *Advances in Ecological Research* **19**: 1-55.

**Eamus D. 1991.** The interaction of rising CO2 and temperatures with water use efficiency. *Plant, Cell & Environment* **14(8)**: 843-852.

**Eisenhauer N. 2012.** Above-belowground interactions as a source of complementarity effects in biodiversity experiments. *Plant Soil* **351**: 1-22.

**Ekblad A, HussDanell K. 1995.** Nitrogen fixation by *Alnus incana* and nitrogen transfer from *A-incana* to *Pinus sylvestris* influenced by macronutrients and ectomycorrhiza. *New Phytologist* **131**(4): 453-459.

**Ellenberg H, Weber HE, Düll R, Wirth V, Werner W, Paulissen D. 1991.** Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica* **18**: 1-248.

**Fischer A, Lindner M, Abs C, Lasch P. 2002.** Vegetation dynamics in central European forest ecosystems (near-natural as well as managed) after storm events. *Folia Geobotanica* **37**(1): 17-32.

**Fornara DA, Tilman D. 2009.** Ecological mechanisms associated with positive diverstiy-productivity relationships in an N-limited grassland. *Ecology* **90**: 408-418.

**Gielen B, Calfapietra C, Sabatti M, Ceulemans R. 2001.** Leaf area dynamics in a closed poplar plantation under free-air carbon dioxide enrichment. *Tree Physiology* **21**(17): 1245-1255.

**Godbold DL, Berntson GM, Bazzaz FA. 1997.** Growth and mycorrhizal colonization of three North American tree species under elevated atmospheric $CO_2$. *New Phytologist* **137**(3): 433-440.

**Haggar JP, Ewel JJ. 1997.** Primary productivity and resource partitioning in model tropical ecosystems. *Ecology* **78**(4): 1211-1221.

**Harper JL. 1977.** *Population biology of plants*. London ; New York: Academic Press.

**Hartig GL. 1791.** *Anweisung zur taxation und beschreibung der forste*. Gessen: Heyer.

**Hattenschwiler S, Handa IT, Egli L, Asshoff R, Ammann W, Korner C. 2002.** Atmospheric CO2 enrichment of alpine treeline conifers. *New Phytologist* **156**(3): 363-375.

**Holtum JAM, Winter K. 2010.** Elevated $CO_2$ and forest vegetation: more a water issue than a carbon issue? *Functional Plant Biology* **37**(8): 694-702.

**Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setala H, Symstad AJ, Vandermeer J, Wardle DA. 2005.** Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* **75**(1): 3-35.

**Hooper DU, Dukes JS. 2004.** Overyielding among plant functional groups in a long-term experiment. *Ecology Letters* **7**: 95-105.

**Hoosbeek MR, Lukac M, Velthorst E, Smith AR, Godbold DL. 2011.** Free atmospheric $CO_2$ enrichment increased above ground biomass but did not affect symbiotic $N_2$-fixation and soil carbon dynamics in a mixed deciduous stand in Wales. *Biogeosciences* **8**(2): 353-364.

**Hughes MK. 1971.** Tree biocontent, net production and litter fall in a deciduous woodland. *Oikos* **22**(1): 62-73.

**Hungate BA, Dijkstra P, Johnson DW, Hinkle CR, Drake BG. 1999.** Elevated $CO_2$ increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Global Change Biology* **5**(7): 781-789.

**Isbell FI, Wilsey BJ. 2011.** Rapid biodiversity declines in both ungrazed and intensely grazed exotic grasslands. *Plant Ecology* **212**(10): 1663-1674.

**Iversen CM. 2010.** Digging deeper: fine-root responses to rising atmospheric $CO_2$ concentration in forested ecosystems. *New Phytologist* **186**(2): 346-357.

**Jacob M, Leuschner C, Thomas FM. 2010.** Productivity of temperate broad-leaved forest stands differing in tree species diversity. *Annals of Forest Science* **67**(5).

**Jolliffe PA. 2000.** The replacement series. *Journal of Ecology* **88**(3): 371-385.

**Karnosky DF, Zak DR, Pregitzer KS, Awmack CS, Bockheim JG, Dickson RE, Hendrey GR, Host GE, King JS, Kopper BJ, Kruger EL, Kubiske ME, Lindroth RL, Mattson WJ, McDonald EP, Noormets A, Oksanen E, Parsons WFJ, Percy KE, Podila GK, Riemenschneider DE, Sharma P, Thakur R, Sober A, Sober J, Jones WS, Anttonen S, Vapaavuori E, Mankovska B, Heilman W, Isebrands JG. 2003.** Tropospheric $O_3$ moderates responses of temperate hardwood forests to elevated $CO_2$: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* **17**(3): 289-304.

**Kelty MJ 1992.** Comparative productivity of monocultures and mixedspecies stands. In: Kelty MJ, Larson BC, Oliver CD eds. *Ecology and Silviculture of Mixed-Species Forests*. Dordrecht, Boston: Kluwer Academic Publishers, 125–141.

**Körner C. 2006.** Plant $CO_2$ responses: an issue of definition, time and resource supply. *New Phytologist* **172**(3): 393-411.

**Körner C, Asshoff R, Bignucolo O, Hattenschwiler S, Keel SG, Pelaez-Riedl S, Pepin S, Siegwolf RTW, Zotz G. 2005.** Carbon flux and growth in mature deciduous forest trees exposed to elevated $CO_2$. *Science* **309**(5739): 1360-1362.

**Kozovits AR, Matyssek R, Blaschke H, Gottlein A, Grams TEE. 2005.** Competition increasingly dominates the responsiveness of juvenile beech and spruce to elevated $CO_2$ and/or $O_3$ concentrations throughout two subsequent growing seasons. *Global Change Biology* **11**(9): 1387-1401.

**Langley JA, Megonigal JP. 2010.** Ecosystem response to elevated $CO_2$ levels limited by nitrogen-induced plant species shift. *Nature* **466**(7302): 96-99.

**Lee TD, Reich, PB, Tjoelker, MG. 2003.** Legume presence increases photosynthesis and N concentrations of co-occurring non-fixers but does not modulate their responsiveness to carbon dioxide enrichment. *Oecologia* **137,** 22-31

**Leuzinger S, Luo Y, Beier C, Dieleman W, Vicca S, Koerner C. 2011.** Do global change experiments overestimate impacts on terrestrial ecosystems? *Trends in Ecology & Evolution* **26**(5): 236-241.

**Loreau M, Hector A. 2001.** Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**(6842): 72-76.

**Lukac M, Calfapietra C, Godbold DL. 2003.** Production, turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated $CO_2$ (POPFACE). *Global Change Biology* **9**(6): 838-848.

**McCarthy HR, Oren R, Finzi AC, Ellsworth DS, Kim H-S, Johnsen KH, Millar B. 2007.** Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric $CO_2$. *Global Change Biology* **13**(12): 2479-2497.

**Miglietta F, Peressotti A, Vaccari FP, Zaldei A, deAngelis P, Scarascia-Mugnozza G. 2001.** Free-air $CO_2$ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* **150**(2): 465-476.

**Millett J, Godbold DL, Smith AR, Grant HO, in press. 2012.** $N_2$ fixation and cycling in *Alnus glutinosa*, *Betula pendula*, and *Fagus sylvatica* woodland exposed to free air $CO_2$ enrichment. *Oecologia*: DOI: 10.1007/s00442-00011-02197-00444.

**Niinemets U. 2006.** The controversy over traits conferring shade-tolerance in trees: ontogenetic changes revisited. *Journal of Ecology* **94**(2): 464-470.

**Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R, De Angelis P, Finzi AC, Karnosky DF, Kubiske ME, Lukac M, Pregitzer KS, Scarascia-Mugnozza GE, Schlesinger WH, Oren R. 2005.** Forest response to elevated $CO_2$ is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences of the United States of America* **102**(50): 18052-18056.

**Norby RJ, Hanson PJ, O'Neill EG, Tschaplinski TJ, Weltzin JF, Hansen RA, Cheng WX, Wullschleger SD, Gunderson CA, Edwards NT, Johnson DW. 2002.** Net primary productivity of a $CO_2$-enriched deciduous forest and the implications for carbon storage. *Ecological Applications* **12**(5): 1261-1266.

**Norby RJ, Sholtis JD, Gunderson CA, Jawdy SS. 2003.** Leaf dynamics of a deciduous forest canopy: no response to elevated $CO_2$. *Oecologia* **136**(4): 574-584.

**Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE. 2010.** $CO_2$ enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences of the United States of America* **107**(45): 19368-19373.

**Okada M, Lieffering M, Nakamura H, Yoshimoto M, Kim HY, Kobayashi K. 2001.** Free-air $CO_2$ enrichment (FACE) using pure $CO_2$ injection: system description. *New Phytologist* **150**(2): 251-260.

**Oksanen E, Sober J, Karnosky DF. 2001.** Impacts of elevated $CO_2$ and/or $O_3$ on leaf ultrastructure of aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) in the Aspen FACE experiment. *Environmental Pollution* **115**(3): 437-446.

**Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schafer KVR, McCarthy H, Hendrey G, McNulty SG, Katul GG. 2001.** Soil fertility limits carbon sequestration by forest ecosystems in a $CO_2$-enriched atmosphere. *Nature* **411**(6836): 469-472.

**Pajtik J, Konopka B, Lukac M. 2011.** Individual biomass factors for beech, oak and pine in Slovakia: a comparative study in young naturally regenerated stands. *Trees-Structure and Function* **25**(2): 277-288.

**Paquette A, Messier C. 2010.** The role of plantations in managing the world's forests in the Anthropocene. *Frontiers in Ecology and the Environment* **8**(1): 27-34.

**Poorter H. 1993.** Interspecific variation in the growth-response of plants to an elevated ambient $CO_2$ concentration. *Vegetatio* **104**: 77-97.

**Pretzsch H 2005.** Diversity and productivity in forests: Evidence from long-term experimental plots. In: SchererLorenzen MKCSED ed. *Forest Diversity and Function: Temperate and Boreal Systems*, 41-64.

**Pretzsch H, Schütze G. 2009.** Transgressive overyielding in mixed compared with pure stands of Norway spruce and European beech in Central Europe: evidence on stand level and explanation on individual tree level. *European Journal of Forest Research* **128**(2): 183-204.

**Reich PB, Falster DS, Ellsworth DS, Wright IJ, Westoby M, Oleksyn J, Lee TD. 2009.** Controls on declining carbon balance with leaf age among 10 woody species in Australian woodland: do leaves have zero daily net carbon balances when they die? *New Phytologist* **183**(1): 153-166.

**Reich PB, Tilman D, Craine J, Ellsworth D, Tjoelker MG, Knops J, Wedin D, Naeem S, Bahauddin D, Goth J, Bengtson W, Lee TD. 2001.** Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric $CO_2$ and N availability regimes? A field test with 16 grassland species. *New Phytologist* **150**(2): 435-448.

**Schlesinger WH, Lichter J. 2001.** Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric $CO_2$. *Nature* **411**(6836): 466-469.

**Schmidt-Vogt H. 1971.** Waschstum und wurzelentwicklung von schwarzerlen verschiedener herkunft. *Allgemeine Forst- und Jagdzeitung* **142**: 149-156.

**Schortemeyer M, Atkin OK, McFarlane N, Evans JR. 2002.** N2 fixation by Acacia species increases under elevated atmospheric $CO_2$. *Plant, Cell & Environment* **25**(4): 567-579.

**Smith AR, Lukac M, Bambrick M, Miglietta F, Godbold DL. 2012.** Tree species diversity interacts with elevated $CO_2$ to induce a greater root system response. *Global Change Biology*: n/a-n/a.

**Teklehaimanot Z, Jones M, Sinclair FL. 2002.** Tree and livestock productivity in relation to tree planting configuration in a silvopastoral system in North Wales, UK. *Agroforestry Systems* **56**(1): 47-55.

**Tilman D, Lehman CL, Thomson KT. 1997.** Plant diversity and ecosystem productivity: Theoretical considerations. *Proceedings of the National Academy of Sciences of the United States of America* **94**(5): 1857-1861.

**Tilman D, Wedin D, Knops J. 1996.** Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* **379**(6567): 718-720.

**Tromp J. 1983.** Nutrient reserves in roots of fruit-trees, in particular carbohydrates and nitrogen. *Plant and Soil* **71**(1-3): 401-413.

**Vila M, Vayreda J, Comas L, Josep Ibanez J, Mata T, Obon B. 2007.** Species richness and wood production: a positive association in Mediterranean forests. *Ecology Letters* **10**(3): 241-250.

**Wardle DA, Nilsson M-C, Gallet C, Zackrisson O. 1998.** An ecosystem-level prespective of allelopath. *Biol Rev* **73**:305-319.

**Weigelt A, Jolliffe P. 2003.** Indices of plant competition. *Journal of Ecology* **91**(5): 707-720.

**Wilson JB. 1988.** Shoot competition and root competition. *Journal of Applied Ecology* **25**(1): 279-296.

**Yasumura Y, Hikosaka K, Matsui K, Hirose T. 2002.** Leaf-level nitrogen-use efficiency of canopy and understorey species in a beech forest. *Functional Ecology* **16**(6): 826-834.

**Zak DR, Pregitzer KS, Kubiske ME, Burton AJ. 2011.** Forest productivity under elevated $CO_2$ and $O_3$: positive feedbacks to soil N cycling sustain decade-long net primary productivity enhancement by $CO_2$. *Ecology Letters* **14**(12): 1220-1226.

**Zhang Y, Chen HYH, Reich PB. 2012.** Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. *Journal of Ecology* **100**(3): 742-749.

## 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 ($^{**}P<0.01$).

| Planting patterm | Species | Diameter | | Height | |
|---|---|---|---|---|---|
| | | Effect | Probability | Effect | Probability |
| *Mono* | *A. glutinosa* | 14% | **0.007**$^{**}$ | 3% | 0.706 |
| | *B. pendula* | 6% | 0.146 | 0% | 0.935 |
| | *F. sylvatica* | 6% | 0.603 | 0% | 0.965 |
| *Poly* | *A. glutinosa* | 4% | 0.618 | 1% | 0.837 |
| | *B. pendula* | 5% | 0.614 | 3% | 0.728 |
| | *F. sylvatica* | -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 | $a$ | $b$ | $R^2$ |
|---|---|---|---|
| *Alnus glutinosa* | 0.5200 | 2.020 | 0.85 |
| *Betula pendula* | 0.4414 | 2.163 | 0.86 |
| *Fagus sylvatica* | 0.6885 | 1.853 | 0.78 |

**Table 3** Effect of $CO_2$ 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 |
|---|---|---|---|---|---|---|
| *Mono* | *A. glutinosa* | ***+29%** | +25% | ***+28%** | ***+32%** | +29% |
| | *B. pendula* | +27% | +13% | +14% | +9% | +16% |
| | *F. sylvatica* | +28% | +33% | +20% | +9% | +22% |
| | | | | | | |
| *Poly* | *A. glutinosa* | +13% | +12% | +3% | +8% | +10% |
| | *B. pendula* | +4% | +8% | +6% | +7% | +6% |
| | *F. sylvatica* | +2% | +5% | +2% | -8% | 0% |

**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 ($^{*}P<0.1$, $^{**}P<0.05$, $^{***}P<0.001$).

| Planting Pattern | Species | Source of Variation | Diameter | | Height | | Biomass | |
|---|---|---|---|---|---|---|---|---|
| | | | F | Probability | F | Probability | F | Probability |
| *Mono* | *A. glutinosa* | 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×treatment | 2.689 | 0.055 | 0.603 | 0.664 | 5.546 | **0.008**$^{**}$ |
| | *B. pendula* | 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×treatment | 0.610 | 0.659 | 0.193 | 0.940 | 0.078 | 0.971 |
| | *F. sylvatica* | 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×treatment | 0.454 | 0.769 | 1.124 | 0.368 | 0.250 | 0.860 |
| *Poly* | *A. glutinosa* | 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×treatment | 0.818 | 0.526 | 0.223 | 0.923 | 0.179 | 0.909 |
| | *B. pendula* | 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×treatment | 0.245 | 0.910 | 0.088 | 0.985 | 0.083 | 0.969 |
| | *F. sylvatica* | 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×treatment | 0.651 | 0.632 | 0.950 | 0.453 | 1.240 | 0.325 |

**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 ($^*P<0.05$).

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

**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).

| Species | 2006[a] | | 2007[b] | | 2008[c] | |
|---|---|---|---|---|---|---|
| | Ambient | FACE | Ambient | FACE | Ambient | FACE |
| *A. glutinosa* | **4.1 ± 0.5** | **3.1 ± 0.2** | 3.4 ± 0.2 | 3.7 ± 0.2 | 4.1 ± 0.0 | 3.9 ± 0.1 |
| *B. pendula* | 3.0 ± 0.1 | 2.7 ± 0.1 | 2.6 ± 0.5 | 2.5 ± 0.2 | 3.7 ± 0.1 | 3.8 ± 0.2 |
| *F. sylvatica* | 2.0 ± 0.1 | 2.0 ± 0.1 | **1.6 ± 0.5** | **3.7 ± 0.1** | 3.0 ± 0.1 | 3.1 ± 0.1 |

**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 ($^{*}P<0.05$, $^{***}P<0.001$)

| | Monoculture | | Polyculture | |
|---|---|---|---|---|
| Source of Variation | 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 $CO_2$ 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.

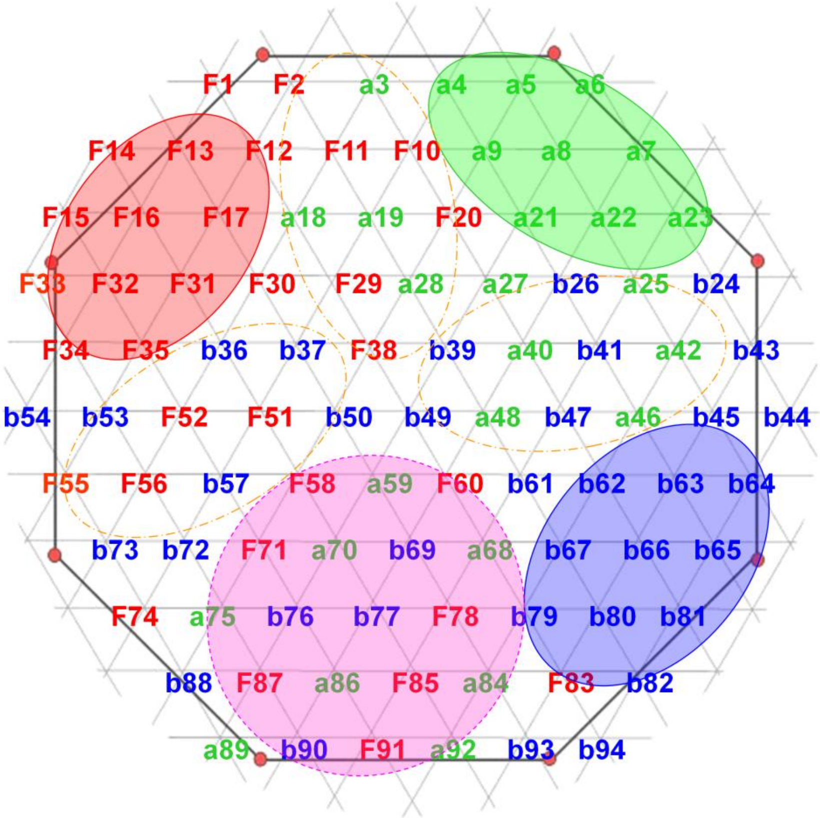

**Fig. 2** Mean ± SE aboveground woody biomass for the species grown in monoculture sub-plots under elevated and ambient $CO_2$ for four years. Aboveground woody biomass was calculated from allometric relationship determined from whole tree harvesting in 2006. Hollow circles indicated elevated atmospheric $CO_2$ and filled circles indicate ambient $CO_2$.

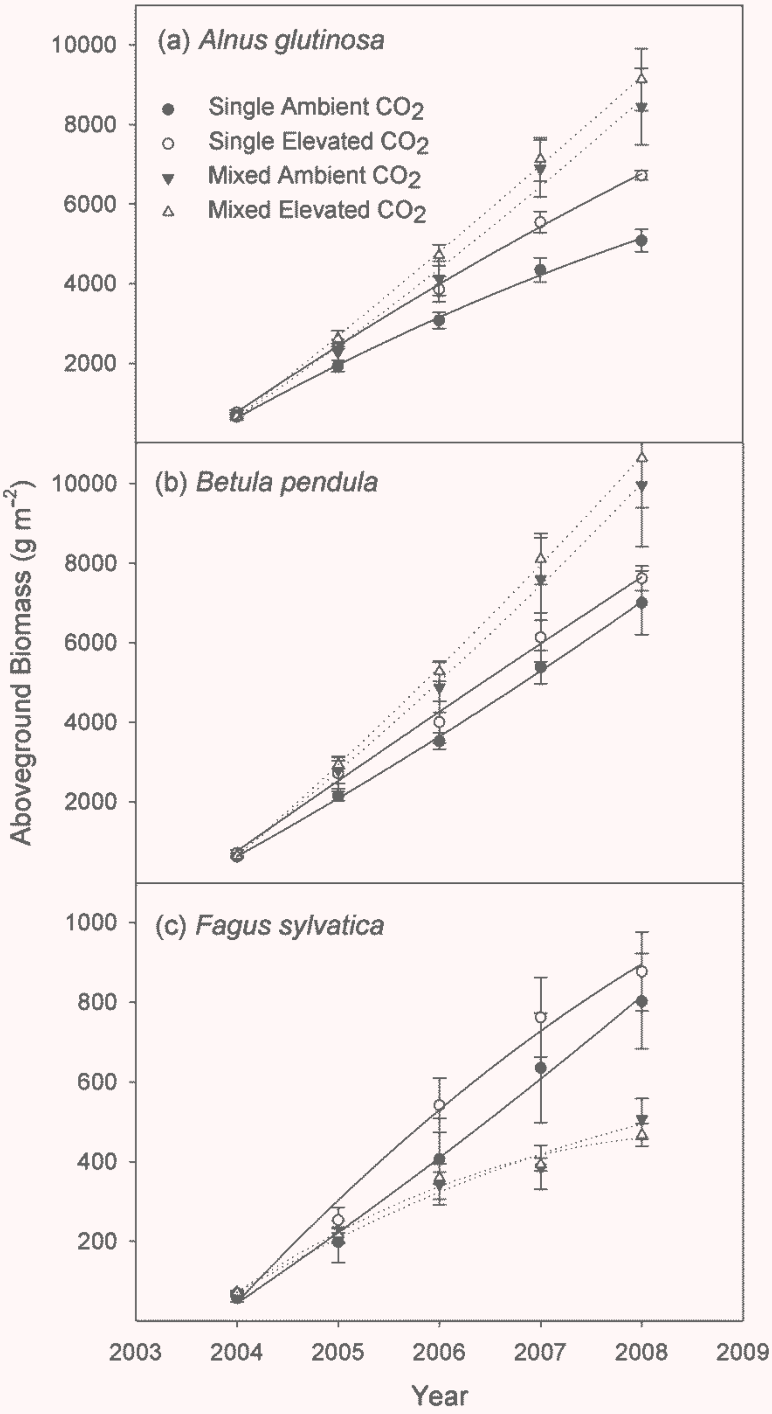

**Fig. 3** Overyielding (a) and $CO_2$ fertilisation (b) effects in pooled data for *A.glutinosa*, *B.pendula* and *F.sylvatica*. Overyielding was calculated as aboveground woody biomass measured in polyculture over that predicted from monocultures. Predicted biomass was calculated by taking 1/3 of biomass observed in each species when grown in monoculture. $CO_2$ fertilisation was calculated as biomass in elevated over ambient $CO_2$ treatments. Values are mean ± SE, *n*=4.

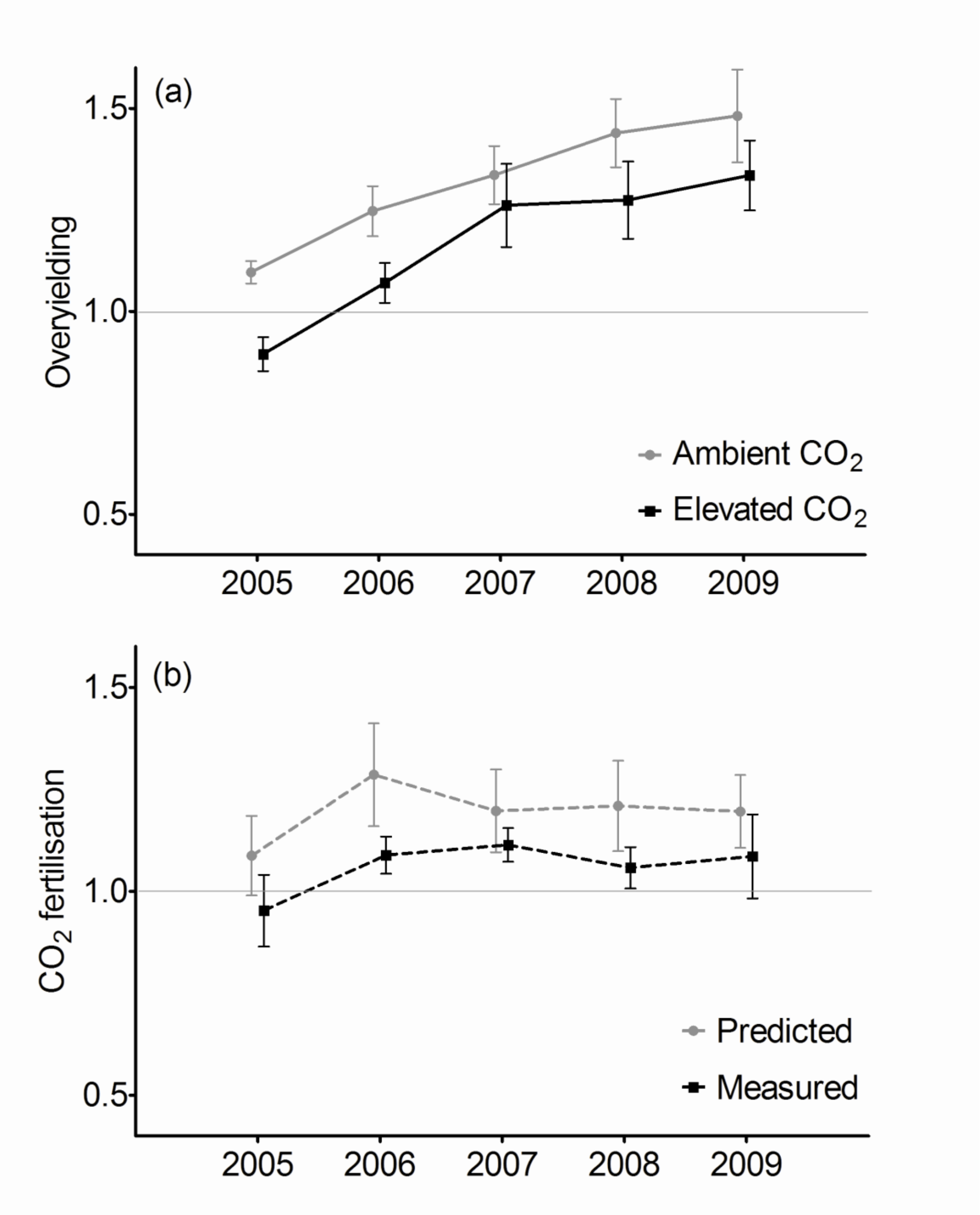

**Fig. 4** Measured leaf area index for *A. glutinosa* and *B. pendula* grown under ambient and elevated $CO_2$ in monoculture (upper panel) and polyculture (lower panel). Values are mean ± SE.

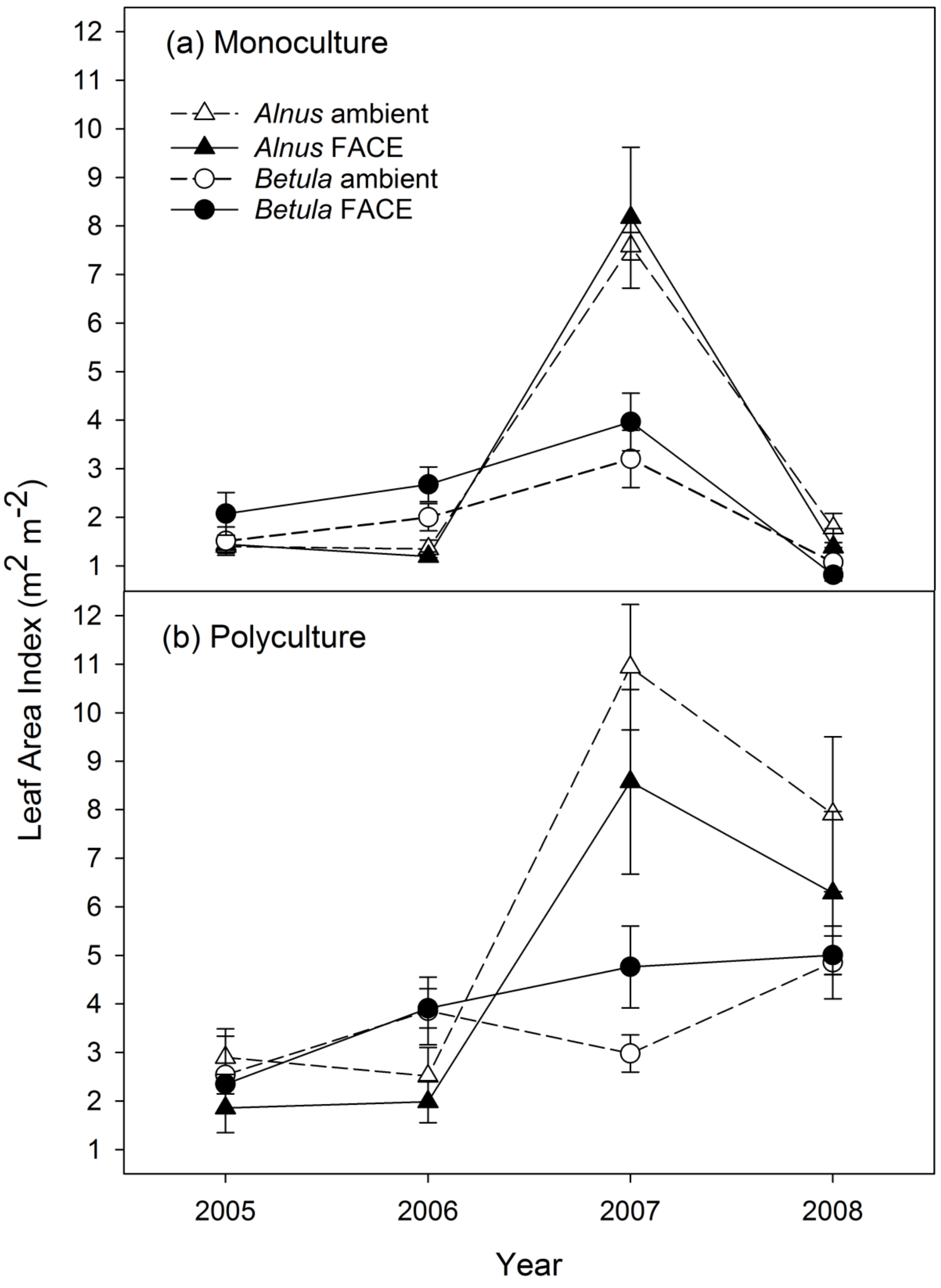

**Fig. 5** Leaf Nitrogen Use Efficiency (NUE) defined as aboveground net primary production per unit of leaf N content. Leaf N Data for (a) *A.glutinosa*, (b) *B.pendula* and (c) *F.sylvatica* are from Ahmed (2006), Anthony (2007) and Millett *et al*. (2011). Values are mean ± SE, *n*=4.

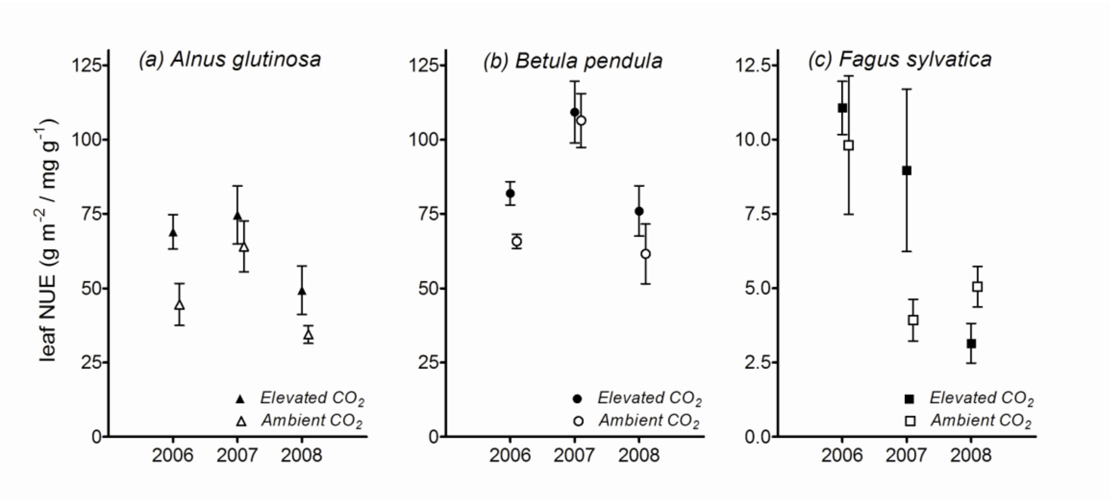

**Fig. 6** Leaf Nitrogen Use Efficiency (NUE), defined as aboveground net primary production per unit of leaf N content, in trees grown in monocultures and a three species mixture. Leaf N Data for (a) *A.glutinosa*, (b) *B.pendula* and (c) *F.sylvatica* are from Ahmed (2006), Anthony (2007) and Millett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