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# 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

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**Abstract.** Through increases in net primary production (NPP), elevated $CO_2$ is hypothesized to increase the amount of plant litter entering the soil. The fate of this extra carbon on the forest floor or in mineral soil is currently not clear. Moreover, increased rates of NPP can be maintained only if forests can escape nitrogen limitation. In a Free atmospheric $CO_2$ Enrichment (FACE) experiment near Bangor, Wales, 4 ambient and 4 elevated [$CO_2$] plots were planted with patches of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica* on a former arable field. After 4 years, biomass averaged for the 3 species was 5497 (se 270) g m$^{-2}$ in ambient and 6450 (se 130) g m$^{-2}$ in elevated [$CO_2$] plots, a significant increase of 17% ($P = 0.018$). During that time, only a shallow L forest floor litter layer had formed due to intensive bioturbation. Total soil C and N contents increased irrespective of treatment and species as a result of afforestation. We could not detect an additional C sink in the soil, nor were soil C stabilization processes affected by elevated [$CO_2$]. We observed a decrease of leaf N content in *Betula* and *Alnus* under elevated [$CO_2$], while the soil C/N ratio decreased regardless of $CO_2$ treatment. The ratio of N taken up from the soil and by $N_2$-fixation in *Alnus* was not affected by elevated [$CO_2$]. We infer that increased nitrogen use efficiency is the mechanism by which increased NPP is sustained under elevated [$CO_2$] at this site.

## 1 Introduction

Using an indirect method, Canadell et al. (2007) estimated the terrestrial carbon (C) sink to account for about a third of total anthropogenic carbon dioxide ($CO_2$) emissions at present. Forest ecosystems are hypothesized to constitute a large part of this sink and to sequester C due to their regrowth and atmospheric $CO_2$ fertilization (Houghton, 2003; Janssens et al., 2003; McMahon et al., 2010). In order to test this hypothesis and to assess the strength of this feedback, Free Air $CO_2$ Enrichment (FACE) experiments in aggrading temperate forests and plantations were initiated. To date, existing experiments have demonstrated that rising atmospheric [$CO_2$] results in increases in net primary production (NPP) and C storage in forest vegetation, e.g. (Calfapietra et al., 2003; DeLucia et al., 1999; Gielen et al., 2005; Hamilton et al., 2002; Handa et al., 2006; Karnosky et al., 2003; Liberloo et al., 2009; Norby et al., 2002). Norby et al. (2005) analyzed the response of NPP (g C m$^{-2}$ y$^{-1}$) to elevated [$CO_2$] in four forest FACE experiments and calculated the following regression $NPP_{elev} = 1.18 \times NPP_{amb} + 55.4$ ($r^2 = 0.97$). The elevated [$CO_2$] effect of 18% was significant ($P < 0.001$), while the positive intercept was not significantly different from zero.

In general, the aboveground biomass contributes its C to the forest litter layer, where it is partially respired and partially incorporated into the mineral soil. Root litter contributes C directly to the mineral soil or, if present, also to the forest litter layer. The extra C taken up due to increased atmospheric [$CO_2$] may also be stored in forest floor litter, organic O and mineral A horizons. Long term C storage is

however thought to primarily take place in mineral soil horizons due to the occurrence of C stabilization mechanisms (Sollins et al., 2006; Six et al., 2002; Von Lützow et al., 2006).

As N availability commonly limits forest productivity, some combination of increased N uptake from the soil and more efficient use of the N assimilated by trees will be necessary to sustain the higher rates of forest NPP at future levels of [$CO_2$]. Based on data from four forest FACE sites, Finzi et al. (2007) demonstrated that increases in N uptake rather than N-use efficiency support high rates of temperate forest productivity under elevated $CO_2$. Nitrogen is also needed for the long term storage of C in stable organic matter fractions in the forest floor and mineral soil. In a meta-analysis based on 65 studies, Van Groenigen et al. (2006) found that soil C content only increases under elevated [$CO_2$] when N is added at rates well above typical atmospheric deposition.

Symbiotic and/or heterotrophic $N_2$-fixation may be a possible source of N to sustain increased N uptake due to high rates of temperate forest productivity under elevated [$CO_2$] (Vitousek et al., 2002). Although assimilation of N by symbiotic $N_2$-fixation is considered to be more costly than uptake of ammonium or nitrate at the plant level, the extra cost might be offset by greater availability of assimilates in high [$CO_2$]. In a growth chamber experiment, elevated [$CO_2$] increased dry weight and total nitrogenase activity of *Robinia pseudoacacia* and *Alnus glutinosa* seedlings, supporting the premise that $CO_2$ enrichment can stimulate symbiotic activity (Norby, 1987). In a chamber experiment with seedlings of *Alnus rubra*, Arnone III and Gordon (1990) observed a positive feedback loop between $N_2$-fixation and photosynthesis in nodulated plants growing under elevated [$CO_2$]. Similarly, in a number of open-top chamber experiments, symbiotic $N_2$-fixing *Alnus glutinosa* trees showed a positive response to elevated [$CO_2$] (Vogel et al., 1997; Temperton et al., 2003).

Hofmockel and Schlesinger (2007) hypothesized that heterotrophic $N_2$-fixation would be enhanced due to increased litter production under elevated [$CO_2$]. Increased N availability to plants would, in turn, meet the additional N required to sustain increased NPP under elevated [$CO_2$]. They conducted series of experiments in which nitrogenase activity was measured in slurries and intact soil cores in response to different levels of substrate, moisture and nutrients. Forest floor and mineral soil samples were obtained from ambient and elevated [$CO_2$] plots at the Duke Forest FACE site. Hofmockel and Schlesinger (2007) did not detect a FACE effect on potential nitrogenase activity and concluded that heterotrophic $N_2$-fixation was not enhanced in temperate pine forests under elevated [$CO_2$].

In 2004 a mixed deciduous forest FACE experiment was initiated near Bangor, Wales, UK. This is the first FACE experiment which includes a symbiotic $N_2$-fixing tree, offering an opportunity to study the effects of elevated [$CO_2$] on $N_2$-fixation in forests. Based on the results by Hofmockel

and Schlesinger (2007) we assumed that heterotrophic $N_2$-fixation at the BangorFACE experiment was not affected by elevated [$CO_2$].

At BangorFACE, preliminary observation showed that biomass growth was increased under elevated [$CO_2$]. Based on these results and published results from other forest FACE experiments, we formulated the following hypotheses:

1. In order to sustain higher rates of forest NPP under elevated [$CO_2$], additional N is taken up from the soil.

2. Elevated [$CO_2$] stimulates symbiotic $N_2$-fixation by increasing C availability in *Alnus glutinosa* root nodules, increasing the ratio of N taken up by $N_2$-fixation to N taken up from the soil.

3. Total soil C content and, to a lesser extent, N content increase due to afforestation.

4. Increased NPP under elevated [$CO_2$] creates additional C storage in the soil.

5. The additional soil C input due to elevated [$CO_2$] results in an increase of coarse, fine and micro-aggregate protected particulate organic matter (POM).

## 2 Methods

The BangorFACE experiment was established at the Henfaes experimental research area in March 2004. The area is located on the coastal plain about 12 km east of Bangor, near the village of Abergwyngregyn, Wales, UK. The climate is Hyperoceanic, with annual rainfall of about 1000 mm. The soil at Henfaes is a fine loamy brown earth over gravel (Rheidol series) classified as a Dystric Cambisol in the FAO system (Teklehaimanot et al., 2002). The parent material consists of postglacial alluvial deposits from the Aber river, comprising Snowdonian rhyolitic tuffs and lavas, microdiorites and dolerite in the stone fractions and Lower Paleozoic shale in the finer fractions. The topography consists of a shallow slope of approximately 1–2° on a deltaic fan. The aspect is northwesterly, at an altitude of 13 to 18 m a.s.l. The depth of the water table ranges between 1 and 6 m.

Trees were planted on two adjacent fields, one of which was previously used both as pasture and arable land, whereas the other was used for small scale agroforestry experiments. The experimental plots were 8 m in diameter, the seedlings of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica* were planted inside the plots at 80 cm spacing in a hexagonal design. The species were planted in a pattern that created mixtures containing one, two and three species. For the purposes of this study, 4 mixtures have been monitored within each experimental plot; three single species sub-plots and a sub-plot containing the mixture of all tree species. The experimental plots were surrounded by a 10 m buffer strip containing the same species and planted at the same density and pattern.

The rest of the plantation was planted with a mixture of tree species at slightly smaller density. In total, 4 ambient and 4 elevated [CO$_2$] plots were randomly located within the plantation in order to form a complete replicated block design. Carbon enrichment started in April 2005 and was achieved by injecting pure CO$_2$ through laser-driller holes in tubing mounted on eight masts (Miglietta et al., 2001). The elevated [CO$_2$], measured at 1 min intervals, was within 30% deviation from the pre-set target concentration of 580 ppm CO$_2$ for 75–79% of the time during the photosynthetically active part of 2005 – 2008. The CO$_2$ used for enrichment originated from natural gas and had a $\delta^{13}$C of −39‰.

## 2.1 Above ground biomass

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 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. The stem diameter measurements were converted to biomass using an allometric model. To develop the model, 8 representative trees for each species were selected to cover a range of diameters and heights. Tree height and stem diameter at 22.5 cm were measured. Regression analysis revealed that height did not significantly contribute to the allometric model. A power regression of stem diameter and woody biomass was used to explain the allometric relationship for each species studied.

## 2.2 Soil sampling

Soil samples were taken from each sub-plot in October of years 2004 through 2008. Bulk density samples were taken at 0–10 cm from the A horizon using a bulk density sampler holding 100 cm$^3$ metal rings. Adjacent to these samples, three bulk samples representative for the 0–10 cm depth were taken with a small spade and mixed for C and N analyses and fractionation. After transportation in a mobile refrigerator, the ring samples were dried at 105 °C for 3 days, while the bulk samples were split in a part that was dried at room temperature and a part that was stored at 4 °C. Bulk densities were calculated based on oven dry weight of the ring samples and ring volume.

Soil texture and pH were only determined for the 2004 samples. After pre-treatment of the samples, the particle size distribution was measured by laser diffraction (Coulter LS230 Grain Sizer; (Buurman et al., 1996). Soil pH was measured with a pH meter (Orion 701A) in a 1 M KCl solution suspension.

For C and N analyses, sub-samples of the air-dried bulk samples were crushed by hand and ball milled after roots were removed. No carbonates were present in the soil. Ammonium and nitrate were measured colorimetrically in a 1M

KCl extraction by using an auto analyzer (Buurman et al., 1996). Total C and N were determined with an elemental analyzer (Interscience EA 1108) and expressed as gram C or N per m$^2$ per depth increment.

## 2.3 Isotope analyses

In 2007, leaves and young branches of *Betula* and *Alnus* and soil samples from all sub-plots were collected, dried, milled and prepared and sent for analysis at the Stable Isotope Laboratory at UC Davis (http://stableisotopefacility.ucdavis.edu). Results were expressed as $\delta^{13}$C (‰) versus the PDB standard and as $\delta^{15}$N (‰) versus standard air. The fraction of soil C derived from litter input (C$_{new}$) between October of 2004 and 2007 ($f_{new\,C}$) was calculated as (Balesdent et al., 1988; Van Kessel et al., 2000):

$$f_{new\,C} = \frac{\left(\delta^{13}C_{soil\,elevated[CO_2]} - \delta^{13}C_{soil\,ambient\,[CO_2]}\right)}{\left(\delta^{13}C_{new} - \delta^{13}C_{soil\,ambient\,[CO_2]}\right)}$$

The new soil C input (g C m$^{-2}$) into the 0–10 cm increment of the elevated [CO$_2$] plots was calculated as:

$$C_{soil\,new} = f_{new\,C} \times C_{soil\,elevated\,[CO_2]}$$

The fraction of N in *Alnus* trees derived from N$_2$-fixation ($f_n$) was calculated as (Amarger et al., 1979; Cadish et al., 2000):

$$f_n = \frac{\left(\delta^{15}N_{birch} - \delta^{15}N_{alder}\right)}{\left(\delta^{15}N_{birch} - B\right)}$$

where birch serves as the non-N$_2$-fixing reference tree and B is a measure of isotopic fractionation during N$_2$-fixation with value −2.6‰ for *Alnus glutinosa* leaves (Domenach et al., 1989).

## 2.4 Physical fractionation

Physical fractionation according to Six et al. (2002) was applied to soil samples in order to measure soil carbon storage. Soil C is stabilized for a relatively longer term within micro-aggregates formed in afforested and forested ecosystems. To quantify micro-aggregate creation, we used a "micro-aggregate isolator", as described by Six et al. (2002), to break up the macro-aggregates while minimizing the break down of the released micro-aggregates. In short, air dried samples were left to slake in deionized water for 5 min. The samples were then poured on top of a 250 μm mesh screen and shaken with 50 glass beads (4 mm diameter). A continuous water flow through the device flushed all released micro-aggregates immediately onto a 53 μm sieve, thus avoiding further disruption. After a complete breakup of macro-aggregates, coarse particulate organic matter (cPOM) and sand remained on the 250 μm mesh screen. The micro-aggregates and the clay and silt sized fraction were separated by a 53 μm sieve. The three obtained fractions, cPOM ( > 250 μm), micro-aggregates and

fine POM (53–250 µm) and the silt and clay sized fraction (<53 µm) were washed into beakers and oven-dried at 50 °C.

The 53–250 µm fraction was further separated into fine POM (light fraction, LF) and micro-aggregates (heavy fraction) by density fractionation. Five gram of dried soil material was suspended in 35 ml of a 1.85 g cm$^{-3}$ sodium polytungstate solution (SPT) in 50 ml conical tubes. The tubes were gently shaken 10 times end over end. Material remaining on the cap and sides of the tubes was rinsed back into solution with more SPT solution and the volume was made up to the 40 ml mark. The tubes were placed under vacuum (−138 kPa) for 10 min. After this, the samples were left to rest for 20 minutes, tubes were balanced with SPT, capped and centrifuged for 60 min at 1250 g. Floating material (LF) was aspirated onto a pre-weighed glass fibre filter, SPT solution was decanted over the filter. The glass fibre filters containing the light fraction were rinsed twice with demineralised water, dried and weighed. The micro-aggregate fraction (heavy fraction, HF) was rinsed twice by adding demineralised water, shook until all material was suspended again and centrifuged. The solution was decanted after centrifugation. Next, the micro-aggregates were dispersed by adding hexametaphosphate (0.5%). After shaking in a reciprocal shaker for about 18 hours, the solution was poured on a 53 µm sieve and washed with deionised water. The micro-aggregate protected POM which remained on the sieve was dried at 50 °C.

## 2.5 Statistical model

The BangorFACE experiment was set up as a replicated split-plot design with four blocks, each containing one ambient and one elevated [$CO_2$] plot. Each plot contained seven sub-plots forming mixtures of one, two or three tree species. The number of replicates per treatment are therefore: $CO_2$ treatment $n = 8$ (4 ambient + 4 elevated [$CO_2$]); Species $n = 32$ (8 *Betula pendula* + 8 *Alnus glutinosa* + 8 *Fagus sylvatica* + 8 mix of the three species).

Two versions of the same general linear model (SPSS 15.0) were used for the analysis of respectively 1) data obtained at one point in time, and 2) data obtained in consecutive years (repeated measures ANOVA). Version 1 was build with the following factors: CO2trmt (fixed), Species (fixed) and Block (random). For version 2 of the model Year (fixed) was added. Main or interaction effects were considered to be significant when the P-value of the F-test was < 0.05.

## 3 Results

### 3.1 Above ground biomass

At the conclusion of the experiment, woody biomass averaged for the tree species was 5497 (se 270) g m$^{-2}$ in ambient and 6450 (se 130) g m$^{-2}$ in elevated [$CO_2$] plots, a significant increase of 17% ($P = 0.018$). The contribution of to-

**Table 1.** Above ground woody biomass after 4 years of free atmospheric $CO_2$ enrichment.

| | Above ground woody biomass (g m$^{-2}$) | | | |
|---|---|---|---|---|
| | ambient [$CO_2$] | | elevated [$CO_2$] | |
| Species | mean | se | mean | se |
| *Alnus* | 7140 | 360 | 8560 | 630 |
| *Betula* | 8750 | 500 | 10190 | 320 |
| *Fagus* | 600 | 70 | 600 | 30 |

tal woody biomass within the elevated [$CO_2$] treatment plots followed the order *Betula* 10190 (se 320) g m$^{-2}$, *Alnus* 8560 (se 630) g m$^{-2}$ and *Fagus* 600 (se 30) g m$^{-2}$ (Table 1). Although not contributing the most to biomass, the largest elevated [$CO_2$] effect was observed in *Alnus* that produced 20% more biomass than in ambient conditions although this was not significant at the 5% level ($P = 0.055$). A significant 16% ($P = 0.046$) increase in woody biomass was observed in *Betula* in response to elevated [$CO_2$] treatment whereas *Fagus* biomass was not altered (−1%; $P = 0.817$).

### 3.2 Initial soil conditions

The soils of all plots classified as "sandy loam" (Soil Survey Division Staff, 1993). Clay, silt and sand percentages were not significantly different between the ambient and elevated [$CO_2$] plots ($P = 0.947, 0.747$ and $0.817$ respectively; Table 2). Soil pH (KCl) was on average 4.6 for both the ambient and elevated [$CO_2$] plots without a significant difference ($P = 0.820$). The initial ammonium concentrations of the ambient and elevated [$CO_2$] plots were 0.09 and 0.09 g N m$^{-2}$ respectively ($P = 0.923$), whereas the nitrate concentrations were 0.72 and 0.84 g N m$^{-2}$ respectively ($P = 0.365$). Initial soil C contents of the ambient and elevated [$CO_2$] plots were respectively 2830 and 2731 g C m$^{-2}$ and not significantly different ($P = 0.492$). Initial soil N contents were not different either ($P = 0.472$) with 258 and 247 g N m$^{-2}$ respectively for ambient and elevated [$CO_2$] plots.

### 3.3 Change of soil C and N

During the experiment, the above ground litter input resulted in an L (almost undecomposed litter less than one year old) forest floor litter layer under most of the plantation. Over the years while taking soil samples, we observed an increasing number of earthworms, their populations probably recovering from the previous use of the site and the field preparation during 2004. In this system, the early phase of litter decomposition (primarily leaching) probably takes place in the L layer, but most of the decomposition then occurs in the top of the mineral soil after the litter had been incorporated into the soil by bioturbation.

**Table 2.** Initial soil conditions at the BangorFACE experimental site.

| CO$_2$ treatment | Clay (%) mean | se | Silt (%) mean | se | Sand (%) mean | se | pH (KCl) mean | se | NH$_4$ (g N m$^{-2}$) mean | se | NO$_3$ (g N m$^{-2}$) mean | se | C$_{total}$ (g C m$^{-2}$) mean | se | N$_{total}$ (g N m$^{-2}$) mean | se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ambient | 9.3 | 0.5 | 28.5 | 1.3 | 62.2 | 1.7 | 4.6 | 0.2 | 0.09 | 0.01 | 0.72 | 0.07 | 2830 | 98 | 258 | 13 |
| elevated | 9.4 | 0.2 | 27.8 | 0.6 | 62.8 | 0.6 | 4.6 | 0.1 | 0.09 | 0.01 | 0.84 | 0.17 | 2731 | 177 | 247 | 16 |
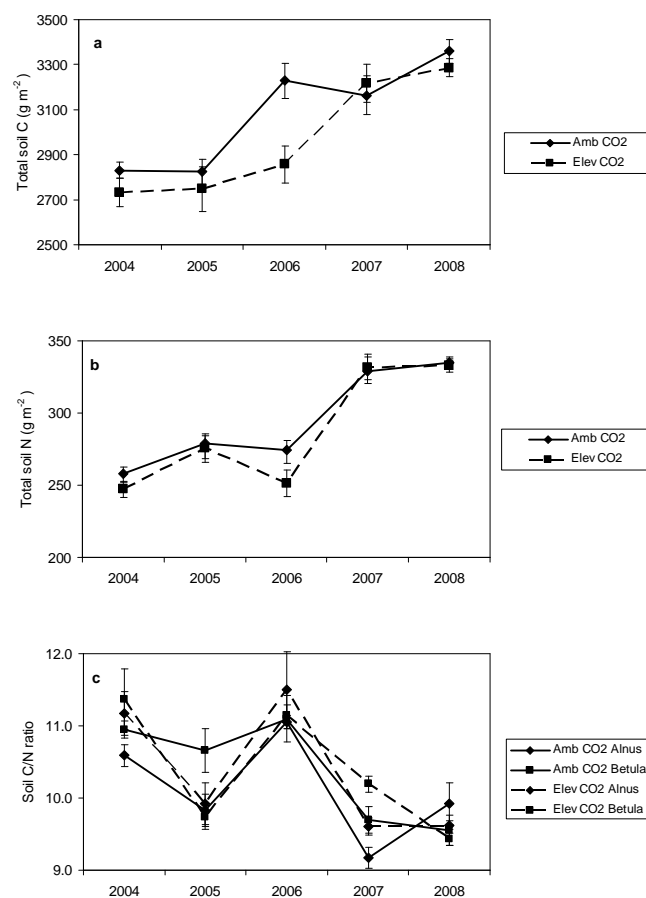


**Fig. 1.** Change of total soil C **(a)** and N **(b)** and soil C/N ratios **(c)** at 0–10 cm depth.

Between October of 2004 and 2008, total soil C content at 0–10 cm depth increased by 530 under ambient and 555 g C m$^{-2}$ under elevated [CO$_2$] (Fig. 1a), whereas total soil N increased by 77 and 86 g N m$^{-2}$, respectively (Fig. 1b). The increase with time was significant for both soil C and N content ($P = 0.005$ and 0.001), but CO$_2$ treatment had no significant effect on C and N content ($P = 0.730$ and 0.767), nor did species have a significant effect ($P = 0.628$ and 0.893). The C/N ratios decreased in 2005, increased in 2006, and decreased again in 2007 ($P = 0.003$; Fig. 1c). Including the N$_2$-fixing species (*Alnus*) did not affect the C/N ratio, i.e. there was no species effect ($P = 0.058$), nor was there a CO$_2$ treatment effect ($P = 0.773$).

Ammonium-N increased under ambient [CO$_2$] throughout the experiment, while under elevated [CO$_2$] we observed the same trend apart from a decrease in 2008 (Fig. 2a). The elevated [CO$_2$] effect was significant ($P = 0.001$), whereas time and species effects were not ($P = 0.092$ and 0.261). Nitrate-N increased during 2006 and 2007 but decreased in 2008 under both ambient and elevated [CO$_2$] (Fig. 2b). CO$_2$ treatment and species did not affect NO$_3$-N ($P = 0.276$ and 0.319), whereas the change with time was significant ($P < 0.001$).

### 3.4 Soil $\delta^{13}$C

Due to the use of CO$_2$ gas with a $\delta^{13}$C value of $-39$‰, the $\delta^{13}$C value of soil C in the top 10 cm of the elevated [CO$_2$] plots decreased from $-27.30$ to $-28.32$‰ during the first three years of fumigation (Fig. 3a). The $\delta^{13}$C values of soil C in the ambient [CO$_2$] plots served as reference values. Based on the decrease of $\delta^{13}$C of soil C in the elevated [CO$_2$] plots, $\delta^{13}$C of litter and $\delta^{13}$C of soil C in the ambient [CO$_2$] plots, we estimated the average input of new soil C into the elevated [CO$_2$] plots to be 494 (se 64) g C m$^{-2}$ between October of 2004 and 2007. The input of new soil C was affected by species ($P = 0.040$) with the lowest input under *Fagus* (Fig. 3b).

### 3.5 N$_2$-fixation

The N concentration in *Alnus* and *Betula* leaves was lower under elevated [CO$_2$] than under ambient [CO$_2$] (Table 3). The $\delta^{15}$N values of leaves of the reference tree (*Betula*) were about equal under ambient and elevated [CO$_2$], i.e. 2.60 and 2.55‰ respectively. These $\delta^{15}$N values represent the uptake of N solely from the soil. Domenach et al. (1989) measured the $\delta^{15}$N of leaves as $-2.6 \pm 0.6$ in *Alnus* grown with atmospheric N$_2$ as the sole source of N, which represents the B value and is the measure of isotopic fractionation during N$_2$-fixation. In *Alnus*, we observed $\delta^{15}$N values of $-0.74$ and $-0.53$‰ respectively under ambient and elevated [CO$_2$]. Based on the sole soil N versus sole N$_2$ source, and the observed $\delta^{15}$N values in *Alnus*, we estimated the fraction of N uptake in *Alnus* through N$_2$-fixation ($f_n$) to be 0.61 under ambient and 0.60 under elevated [CO$_2$]. This $f_n$ fraction was not affected by [CO$_2$] ($P = 0.747$).

**Table 3.** N concentration and $\delta^{15}$N of *Alnus* and *Betula* leaves and the fraction of N in *Alnus* taken up through N$_2$-fixation ($f_n$).

| $CO_2$ treatment | Species | $\mu$gN g$^{-1}$ dw | | $\delta^{15}$N | | $f_n$ | |
|---|---|---|---|---|---|---|---|
| | | mean | se | mean | se | mean | se |
| ambient [$CO_2$] | *Alnus* | 71.36 | 2.34 | −0.74 | 0.16 | 0.61 | 0.02 |
| | *Betula* | 64.05 | 1.84 | 2.60 | 0.10 | | |
| elevated [$CO_2$] | *Alnus* | 60.48 | 3.41 | −0.53 | 0.13 | 0.60 | 0.02 |
| | *Betula* | 56.86 | 4.04 | 2.55 | 0.10 | | |

**Table 4.** C and N contents of isolated soil fractions.

| Soil C fraction | $CO_2$ treatment | Species | g C m$^{-2}$ | | g N m$^{-2}$ | |
|---|---|---|---|---|---|---|
| | | | mean | se | mean | se |
| coarse POM > 250 $\mu$m | ambient [$CO_2$] | *Alnus* | 788 | 173 | 64 | 15 |
| | | *Betula* | 740 | 124 | 47 | 10 |
| | | *Fagus* | 727 | 122 | 45 | 11 |
| | | mix | 673 | 93 | 47 | 7 |
| | elevated [$CO_2$] | *Alnus* | 993 | 196 | 64 | 29 |
| | | *Betula* | 941 | 42 | 64 | 13 |
| | | *Fagus* | 761 | 120 | 48 | 4 |
| | | mix | 812 | 135 | 41 | 3 |
| fine POM 53–250 $\mu$m | ambient [$CO_2$] | *Alnus* | 158 | 38 | 13 | 5 |
| | | *Betula* | 131 | 16 | 11 | 2 |
| | | *Fagus* | 138 | 21 | 12 | 2 |
| | | mix | 149 | 31 | 10 | 2 |
| | elevated [$CO_2$] | *Alnus* | 168 | 19 | 13 | 3 |
| | | *Betula* | 224 | 43 | 17 | 3 |
| | | *Fagus* | 158 | 12 | 14 | 1 |
| | | mix | 223 | 46 | 18 | 3 |
| micro-aggregate protected POM 53–250 $\mu$m | ambient [$CO_2$] | *Alnus* | 504 | 59 | 32 | 6 |
| | | *Betula* | 610 | 26 | 43 | 5 |
| | | *Fagus* | 536 | 54 | 47 | 7 |
| | | mix | 536 | 61 | 38 | 4 |
| | elevated [$CO_2$] | *Alnus* | 428 | 54 | 27 | 8 |
| | | *Betula* | 485 | 102 | 44 | 17 |
| | | *Fagus* | 439 | 42 | 30 | 6 |
| | | mix | 442 | 66 | 31 | 10 |

## 3.6 Soil organic matter fractionation

Averaged over the species, the coarse POM C fraction was larger under elevated [$CO_2$] than under ambient [$CO_2$], 877 and 732 g C m$^{-2}$ respectively, however this effect was not significant ($P = 0.356$, Table 4). The coarse POM N fractions were about equal under ambient and elevated [$CO_2$] ($P = 0.928$). Neither coarse POM C or N fractions were affected by species ($P = 0.230$ and $P = 0.067$).

Similarly to coarse POM, the fine POM C fraction was larger under elevated than under ambient [$CO_2$], 193 and 144 g C m$^{-2}$ respectively while this effect was again not significant ($P = 0.138$). However, the fine POM N fraction was significantly larger under elevated than under ambient [$CO_2$], i.e. 16 and 11 g N m$^{-2}$ ($P = 0.041$). The fine POM C and N fractions were not affected by species ($P = 0.650$ and $P = 0.950$).

The micro-aggregate protected POM C fraction was larger under ambient [$CO_2$] than under elevated [$CO_2$], i.e 547 and 449 g C m$^{-2}$, but not significantly ($P = 0.200$). The micro-aggregate protected POM N fraction was also larger under ambient $CO_2$ (40 and 33 g C m$^{-2}$; $P = 0.314$), however,
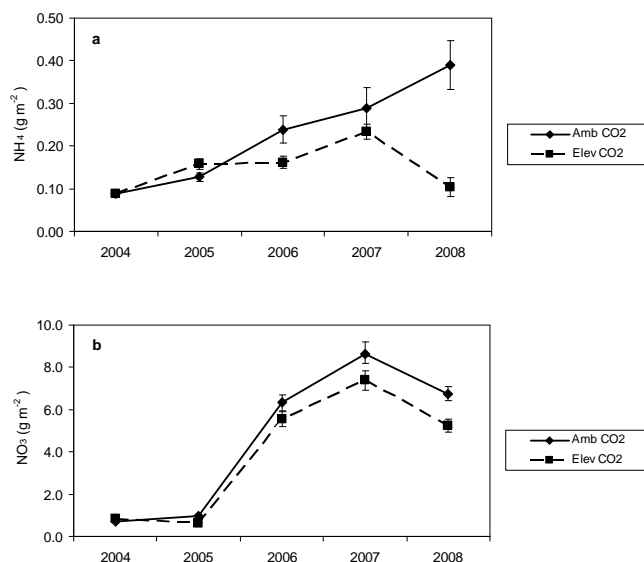
**Fig. 2.** Change of NH$_4$-N (**a**) and NO$_3$-N (**b**) at 0–10 cm depth.



**Fig. 3.** Change of soil $\delta^{13}$C (**a**) and new soil C (**b**) in elevated [CO$_2$] plots at 0–10 cm depth between October of 2004 and 2007.

again not significantly. Just like the coarse and fine POM fractions, the micro-aggregate protected POM C and N fractions were not affected by species either ($P = 0.564$ and $P = 0.244$).

## 4 Discussion

### 4.1 Above ground biomass

Norby et al. (2005) calculated an average CO$_2$ response of 18% based on NPP (g C m$^{-2}$ y$^{-1}$) data of four forest FACE experiments. Despite the fact that the observed above ground woody biomass data can not be compared directly to the NPP data, the response ratios (expressed as response percentage) may be related since the annual woody biomass increment (after conversion to g C m$^{-2}$) is one of the largest increments that make up NPP. The average CO$_2$ response of 17% observed at BangorFACE, based on above ground woody biomass, is close to the average NPP-based CO$_2$ response of the other forest FACE experiments.

### 4.2 Change of soil C

The increase of total C in the top 10 cm of the mineral soil during the four year experiment was about equal under ambient and elevated [CO$_2$], reaching 530 and 555 g C m$^{-2}$ respectively, which makes the expected additional C sink under elevated [CO$_2$] negligible and, in this experiment, insignificant. Similarly, we did not observe any species effect on soil C, which may in part have been obscured by wind redistribution of above ground litter between the patches of different tree species. In 2007, cross contamination with leaves from other species was about 24–27% of the total litterfall within
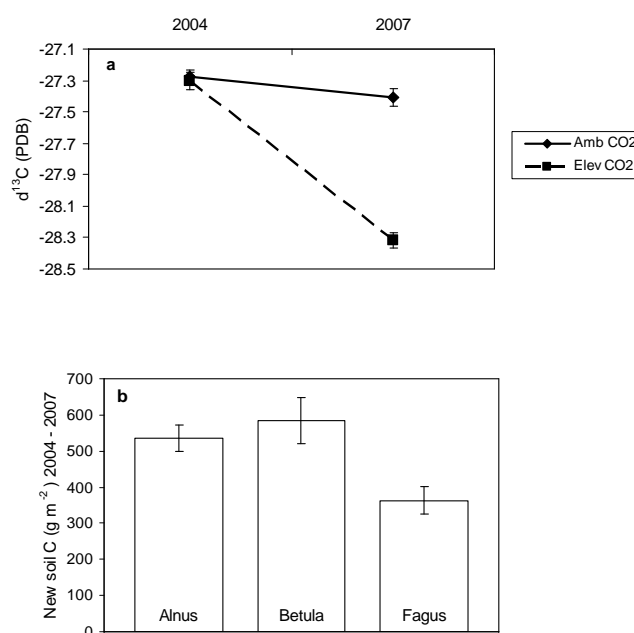
*Alnus* and *Betula* single species patches (Smith, 2010). The *Fagus* leaves remained on branches until the following spring and then slowly shed. The observed increase of soil C, irrespective of treatment, is therefore due to afforestation of the former agricultural fields. Based on the $\delta^{13}$C data we estimated the average input of new soil C into the elevated [CO$_2$] plots to be 494 (se 64) g C m$^{-2}$ between October of 2004 and 2007. This input seems to relate well to the average increase of total soil C in the elevated [CO$_2$] plots over the same period, i.e. 486 g C m$^{-2}$ (Fig. 1a).

Carbon storage in litter and soil has been assessed at several other forest FACE experiments. For instance, at the Duke Forest and POP-EuroFACE experiments a significant additional C sink was created in the litter layer after six years of elevated CO$_2$ treatment (Table 5) (Lichter et al., 2005; Hoosbeek and Scarascia-Mugnozza, 2009). However, in Duke Forest the stimulation of organic matter accumulation by elevated [CO$_2$] ceased after the sixth year, resulting in an average additional C sink of ~30 g C m$^{-2}$ yr$^{-1}$ over the nine year experiment (Lichter et al., 2008). At both sites, characterized by negligible bioturbation, the increase of C in the mineral soil depended solely on C input from roots and on downward leaching of DOC from the litter layers. In these forests it was not enhanced by elevated [CO$_2$], i.e. no significant additional C sink was created in the mineral soil. At the Oak Ridge FACE experiment, most of the above ground litter was incorporated into the mineral soil by bioturbation. This resulted, in combination with C input from root turnover, in a significant additional C sink in the top 5 cm of the mineral soil (Jastrow et al., 2005). At the 0–15 cm increment,

**Table 5.** Additional C sinks in the forest floor – soil systems and major vegetation and soil characteristics of selected forest FACE experiments (Scarascia-Mugnozza et al., 2006; Schlesinger et al., 2006; Norby et al., 2006; Norby et al., 2005; Finzi et al., 2007; Hoosbeek and Scarascia-Mugnozza, 2009; Lichter et al., 2008).

| | Duke Forest | Oak Ridge | POP-EuroFACE | BangorFACE |
|---|---|---|---|---|
| Type of vegetation | coniferous | deciduous | deciduous | deciduous |
| NPP ($g DM m^{-2} y^{-1}$) (ambient-elevated CO$_2$) | 1400–1800 | 2100–2600 | 3100–3800 | 1112–1225 |
| Forest floor FACE C sink ($g C m^{-2} y^{-1}$) | 52* (yrs 1–6) 30 (yrs 1–9) | | 32 * | |
| Mineral soil FACE C sink ($g C m^{-2} y^{-1}$; soil depth) | 27 (0–15 cm) | 44* (0–5 cm) 28 (0–15 cm) | 1 (0–10 cm; yrs 1-6) 54 (0–10 cm; yrs 4–6) | 6 (0–10 cm) |
| Soil classification (USDA) | Ustic Hapludalf | Aquic Hapludult | Pachic Xerumbrept | Fluventic Dystrochrept |
| Soil texture | clay loam | silty clay loam | loam and silt loam | sandy loam |
| Relative soil fertility | low | intermediate | high | high |
| Soil pH | 5.75 | 5.5–6.0 | 4.8–5.0 | 4.1–5.1 |
| Base saturation | low | high | low | high |
| Vertical mixing – bioturbation | no | yes | negligible | yes |
| Mechanism to sustain NPP under FACE | increased N uptake from the soil | increased N uptake from the soil | increased N-use efficiency | increased N-use efficiency |

* indicates significant ($P < 0.05$) FACE effect.

however, this elevated [CO$_2$] effect on soil C was no longer significant. Mixing of above ground litter into the mineral soil by bioturbation may have facilitated the elevated [CO$_2$] effect at the 0–5 cm increment. At the BangorFACE experiment, we also observed bioturbation which resulted in the "concentration" of above and below ground litter inputs in the top of mineral soil. However, at the BangorFACE site this "concentration effect" did not amplify a possible elevated [CO$_2$] effect on soil C storage.

### 4.3 Soil organic matter stabilization

In order to evaluate the effect of elevated [CO$_2$] on soil C dynamics it is not sufficient to only look at changes in total C content, but it is also necessary to assess possible soil C stabilization mechanisms. The stability of SOM is controlled by the chemical structure of the organic matter and the existence of protection offered by the soil matrix and minerals (Baldock and Skjemstad, 2000; Krull et al., 2003; Davidson and Janssens, 2006). Oades (1993) suggested a model of aggregate formation in which micro-aggregates ($\sim$100 µm in diameter) are formed within macro-aggregates ($> 250$ µm in diameter). Fresh litter entering the soil forms sites for microbial activity and nucleation centers for aggregation (Six et al., 2002). This fraction is, in the conceptual model of aggregate formation, represented by coarse POM ($> 250$ µm). We observed that C and N contents of this coarse POM fraction were not significantly larger under elevated [CO$_2$], meaning that the first phase towards SOM stabilization was not significantly enhanced under elevated [CO$_2$].

As the organic matter enclosed in the macro-aggregates is decomposed, fine POM and micro-aggregates (53–250 µm) are formed. The fine POM C fraction was not affected by CO$_2$ treatment or species, implying that the next step towards stabilization was also unchanged. However, the fine POM N fraction was significantly larger under elevated [CO$_2$], suggesting increased microbial activity and N-immobilization. At the smallest scale, the micro-aggregate protected C and N fractions were also not affected by CO$_2$ treatment and species.

The degree of soil C stabilization was found to vary among FACE experiments with trees. At Duke Forest, the increase of soil C due to forest regrowth occurred entirely within the free light fraction, while the iPOM and mineral associated fractions were not affected by elevated [CO$_2$] (Lichter et al., 2005). No additional soil C protection and stabilization took place. At Oak Ridge, the protection and stabilization processes in the soil kept up with the extra C input under elevated [CO$_2$], i.e. the additional C input due to elevated [CO$_2$] was protected at the same rate as under ambient [CO$_2$] (Jastrow et al., 2005). At POP-EuroFACE, iPOM and mineral associated C and N fractions increased in macro-aggregates and in newly formed micro-aggregates which indicates that protection and stabilization processes increased due to elevated [CO$_2$] (Hoosbeek and Scarascia-Mugnozza, 2009). However, at BangorFACE, we observed no elevated [CO$_2$] or species effect on soil C stabilization mechanisms, leading us to conclude that soil C stabilization processes were not affected by CO$_2$ treatment or by species.

## 4.4 Soil N and N uptake

Total soil N was not affected by CO$_2$ treatment or species and nor was the C/N ratio. However, the interannual variation of soil C and N did not follow the same pattern. In 2004 and 2005 the NPP was still relatively low, but increased in 2006. As a result, in 2006 soil C increased both under ambient and elevated [CO$_2$], however, the increase under elevated [CO$_2$] was smaller. At the same time, soil N did not change under ambient [CO$_2$] while there was a decrease under elevated [CO$_2$]. Based on a increased biomass production under elevated [CO$_2$], we expected a reverse scenario, i.e. a larger increase of soil C and N under elevated [CO$_2$]. However, the extra biomass under elevated [CO$_2$] could have resulted in a larger availability of labile substrate in the soil, which may have caused a priming effect. Priming may occur in forest FACE experiments established on former agricultural soils. For instance, Hoosbeek et al. (2004) observed a priming effect during the second and third year of a FACE experiment with poplar trees established on former agricultural soils in central Italy. This temporal priming effect and the associated loss of older SOM was driven by the experiment, i.e. higher labile C availability due to elevated [CO$_2$], and by the change of land use, i.e. going from a high fertility agricultural soil to a forest soil with a declining soil fertility status (Hoosbeek et al., 2006; Hoosbeek and Scarascia-Mugnozza, 2009). In a scrub-oak ecosystem with higher biomass production under elevated [CO$_2$], Carney et al. (2007) observed a decline of soil C due to relative higher abundances of fungi and higher activities of phenol oxidase which is responsible for the degradation of recalcitrant SOM such as lignin. Based on incubation experiments at the Duke FACE experiment, Billings and Ziegler (2008) inferred that increasing N limitation under elevated [CO$_2$] would result in greater turnover rates of relatively stable soil C pools.

At BangorFACE we observed that total soil C increased less and total N decreased more under elevated than under ambient [CO$_2$] during the second year. We infer that soil microbial populations increased under elevated [CO$_2$] (higher labile C availability) and more available N was taken up by the microbial populations from this former agricultural soil (lower NH$_4^+$ concentration under elevated [CO$_2$] in 2006). In a next step, or in addition, the extended microbial population decomposed N-rich older SOM making N available to the microbial population and plants. Since this source of N requires more energy, the population will decrease and adjust itself to the new availability of substrate and nutrients. After the increased decomposition of SOM diminished, concentrations of NH$_4^+$ and NO$_3^-$ will also go down again and adjust to levels that fit the new nutrient status of a young forest soil. This is in line with our observations, i.e. NH$_4^+$ decreased after 2007 under elevated [CO$_2$], while in the ambient [CO$_2$] plots NH$_4^+$ did not decrease yet. While NO$_3^-$ decreased both under ambient and elevated [CO$_2$] with lower concentrations under elevated [CO$_2$]. We postulate that the priming effect and subsequent lowering of soil NH$_4^+$ and NO$_3^-$ concentrations (not observed yet for NH$_4^+$ in the ambient [CO$_2$] plots) are largely due to the change of land use and that these transitional processes were enhanced under elevated [CO$_2$].

The N concentration in *Alnus* and *Betula* leaves was lower under elevated [CO$_2$] than under ambient [CO$_2$], which means that the demand for more N in order to sustain higher biomass productivity under elevated [CO$_2$] was at least in part met by an increase of the N-use efficiency (NUE). A similar effect was observed by Calfapietra et al. (2007) in three *Populus* species in at the POP-EuroFACE experiment in central Italy.

Since *Alnus* supports symbiotic N$_2$-fixation, we hypothesized that it would be able to gain extra N by increasing the C supply to N$_2$-fixing bacteria. We did not observe this effect, the N uptake ratio (N$_2$-fixation/soil N) did not change in high [CO$_2$] treatment. *Alnus* growing in elevated [CO$_2$] did not use the extra available biomass (labile C) to increase symbiotic N$_2$-fixation in order to meet the higher N demand under higher productivity. Instead, *Alnus* increased its NUE.

Finzi et al. (2007) pointed out that some combination of increased N uptake from the soil and more efficient use of the N already assimilated by trees is necessary to sustain the high rates of forest NPP under elevated [CO$_2$]. Based on a larger FACE data set including a wider variety of plants, Leakey et al. (2009) concluded that elevated [CO$_2$] increases NUE. At Oak Ridge, the elevated [CO$_2$] induced soil C accrual was accompanied by a significant increase in soil N, i.e. elevated [CO$_2$] did not affect the C/N ratio of the mineral soil. Jastrow et al. (2005) postulated that elevated [CO$_2$] also affected N cycling by some combination of reducing N losses, stimulation of N fixation and increasing N uptake through greater root exploration. During the first three years of the POP-EuroFACE experiment, the increase of NUE was the major mechanism sustaining increased NPP under elevated [CO$_2$] Calfapietra et al., 2007). As mentioned before, leaf N content of *Alnus* and *Betula* decreased under elevated [CO$_2$] while the soil C/N ratio was not affected by elevated [CO$_2$]. From this we infer that at Bangor the major mechanism to sustain increased NPP under elevated [CO$_2$] is also based on increased NUE. This fits well with the conclusions of (Finzi et al., 2007) (Table 5) stating that on the one hand, at sites with N-limited growth, i.e. Duke Forest and Oak Ridge, trees increase N uptake from the soil supporting greater NPP, while on the other hand, at sites without N-limitation, i.e. POP-EuroFACE and BangorFACE established on former agricultural soils, increased N-use efficiency seems to be the major mechanism sustaining increased NPP under elevated [CO$_2$].

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