Biogeosciences Discuss., 7, 4153–4180, 2010
www.biogeosciences-discuss.net/7/4153/2010/
doi:10.5194/bgd-7-4153-2010

Biogeosciences Discussions

Free atmospheric CO_2 enrichment did not affect symbiotic N_2-fixation and soil carbon dynamics in a mixed deciduous stand in Wales

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Received: 28 April 2010 – Accepted: 24 May 2010 – Published: 4 June 2010

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Through increases in net primary production (NPP), elevated CO_2 is hypothesizes 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 CO_2 and 4 FACE plots were planted with patches of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica* on a former arable field. Four years after establishment, 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 FACE. We observed a decrease of leaf N content in *Betula* and *Alnus* under FACE, 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 FACE. 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 concentrations result in increases in net primary production (NPP) and C storage in forest vegetation, e.g.

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(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 to elevated CO_2 in four forest FACE experiments and found that its response is well conserved across a broad range of productivity, with a stimulation at the median of $23 \pm 2\%$.

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 concentrations may also be stored in forest floor litter, organic O and mineral A horizon. 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.

Biological 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 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*

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rubra, Arnone III and Gordon (1990) observed a positive feedback loop between N-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).

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 fixation in forests. Taking into account preliminary biomass growth observations and published results from other forest FACE experiments, we formulated the following hypotheses:

1. In order to sustain higher rates of forest NPP under FACE, additional N is taken up from the soil.
2. FACE 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 FACE creates additional C storage in the soil.
5. The additional soil C input due to FACE 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

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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 FACE 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 concentrations, measured at 1 min 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 part of 2005–2008. The CO_2 used for enrichment originated from natural gas and had a $\delta^{13}C$ of −39‰.

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

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and N analyses and fractionation. After transportation in a mobile refrigerator, the ring samples were dried at 105 °C for 3 d, 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 1 M 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.

2.1 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 δ^{13}C (‰) versus the PDB standard and as δ^{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):

$$f_{new\,C} = \frac{\left(\delta^{13}C_{soil\,FACE} - \delta^{13}C_{soil\,ambient}\right)}{\left(\delta^{13}C_{new} - \delta^{13}C_{soil\,ambient}\right)} \quad (1)$$

The new soil C input (g C m^{-2}) into the 0–10 cm increment of the FACE plots was calculated as:

$$C_{soil\,new} = f_{new\,C} \cdot C_{soil\,FACE} \quad (2)$$

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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):

$$f_n = \frac{\left(\delta^{15}N_{\text{birch}} - \delta^{15}N_{\text{alder}}\right)}{\left(\delta^{15}N_{\text{birch}} - B\right)} \quad (3)$$

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).

2.2 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 grams of dried soil material were suspended in 35 ml of a 1.85 g cm^{-3} sodium polytungstate solution (SPT)

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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 min, 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 h, 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.

2.3 Statistical model

The BangorFACE experiment was set up as a replicated split-plot design with four blocks, each containing one control and one FACE plot. Each plot contained seven sub-plots forming mixtures of one, two or three tree species. The numbers of replicates per treatment are therefore: CO_2 treatment n=8 (4 ambient+4 FACE); 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.

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3 Results

3.1 Initial soil conditions

Soil clay percentage varied between 8 and 10% among all plots, whereas silt varied between 25 and 33% (Table 1). These textural differences are relatively minor and the soils of all plots were well within the "sandy loam" textural class (Soil_Survey_Division_Staff, 1993). The initial soil pH ranged between 4.1 and 5.1 among the plots, however, the mean pH of control and FACE plots was about equal, i.e. 4.6 (s.e. 0.2) and 4.6 (0.1), respectively. The initial ammonium concentrations of the control and FACE plots were 0.09 (0.00) and 0.09 (0.00) g N m^{-2}, respectively, whereas the nitrate concentrations were 0.72 (0.05) and 0.84 (0.12) g N m^{-2}, respectively. These concentrations were not significantly different at the plot, block or CO_2 treatment level.

The initial soil C and N contents of the plots were significantly different, ranging between 2171 (269) and 3191 (74) g C m^{-2} and 214 (8) and 304 (8) g N m^{-2}. However, averaged for the CO_2 treatments, the initial differences did not differ significantly, i.e. 2830 (69) and 2731 (125) g C m^{-2} and 258 (9) and 247 (11) g N m^{-2} respectively for control and FACE plots.

3.2 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.

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Between October of 2004 and 2008, total soil C content at 0–10 cm depth increased by 530 under ambient CO_2 and 555 g C m^{-2} under FACE (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 C and N, but the CO_2 treatment and species effects were not significant. The C/N ratios decreased in 2005, increased in 2006, and decreased again in 2007 (Fig. 1c). Including the N_2-fixing species (*Alnus*) did not affect the C/N ratio, i.e. there was no species effect, nor was there a CO_2 treatment effect.

Ammonium-N increased under ambient CO_2 throughout the experiment, while under FACE we observed the same trend apart from a decrease in 2008 (Fig. 2a). The FACE effect was significant, whereas time and species effects were not. Nitrate-N increased during 2006 and 2007 but decreased in 2008 under both ambient CO_2 and FACE (Fig. 2b). CO_2 treatment and species did not affect NO_3-N, whereas the change with time was significant.

3.3 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 FACE 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 FACE 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 FACE plots to be 494 (se 64) g C m^{-2} between October of 2004 and 2007. The largest input of new soil C took place under *Alnus* and *Betula*, although this species effect was not significant (Fig. 3b).

3.4 N_2-fixation

The N concentration in *Alnus* and *Betula* leaves was lower under FACE than under ambient CO_2 (Table 2). The $\delta^{15}N$ values of leaves of the reference tree (*Betula*) were about equal under ambient CO_2 and FACE, i.e. 2.60 (se 0.10) and 2.55‰ (se 0.10),

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respectively. These δ^{15}N values represent the uptake of N solely from the soil. Domenach et al. (1989) measured the δ^{15}N of leaves as -2.6 ± 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 δ^{15}N values of −0.74 (se 0.16) and −0.53‰ (se 0.13) respectively under ambient CO_2 and FACE. Based on the sole soil N versus sole N_2 source, and the observed δ^{15}N values in *Alnus*, we estimated the fraction of N uptake in *Alnus* through N_2-fixation (f_n) to be 0.61 (se 0.02) under ambient and 0.60 (se 0.02) under elevated CO_2.

3.5 Soil organic matter fractionation

Averaged over the species, the coarse POM C fraction was larger under FACE than under ambient CO_2, 877 and 732 g C m^{-2}, respectively, however this effect was not significant (P=0.356, Table 3). The coarse POM N fractions were about equal under ambient CO_2 and FACE (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 FACE than under ambient CO_2, 193 and 144 g C m^{-2} respectively and this effect was also not significant (P=0.138). However, the fine POM N fraction was significantly larger under FACE 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 FACE, i.e. 547 and 449 g C m^{-2} (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, again not significantly. Just like the POM fractions, the micro-aggregate protected POM C and N fractions were not affected by species either (P=0.564 and P=0.244).

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4 Discussion

4.1 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 CO_2 and FACE, 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, based on litterfall data published elsewhere, was about 24–27% of the total litterfall within alder and birch single species patches. The beech 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 FACE 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 FACE 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 4) (Lichter et al., 2005; Hoosbeek and Scarascia-Mugnozza, 2009). However, in Duke Forest the stimulation of organic matter accumulation by FACE 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 FACE, 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

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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, however, this FACE effect on soil C was no longer significant. Hoosbeek and Scarascia-Mugnozza (2009) hypothesized that the mixing of above ground litter into the mineral soil by bioturbation may have facilitated the FACE 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 at the top of mineral soil. However, at the BangorFACE site this "concentration effect" did not amplify a possible FACE effect on soil C storage.

4.2 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 (~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 FACE, meaning that the first phase towards SOM stabilization was not significantly enhanced under FACE.

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

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FACE, 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 FACE (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 FACE, i.e. the additional C input due to FACE 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 FACE (Hoosbeek and Scarascia-Mugnozza, 2009). However, at BangorFACE, we observed no FACE 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.

4.3 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 CO_2 and FACE, however, the increase under FACE was smaller. At the same time, soil N did not change under ambient CO_2 while there was a decrease under FACE. Based on a higher NPP under FACE, we expected a reverse scenario, i.e. a larger increase of soil C and N under FACE. However, the extra NPP under FACE could have resulted in a larger availability of labile substrate in the soil, which may have caused a priming effect (Hoosbeek et al., 2004). This priming effect may have stimulated the decomposition of older SOM with a relatively low C/N ratio, which may explain the observed limited increase of soil C and a decrease of

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N in 2006 under FACE. Usually, a priming effect is a temporal phenomenon, as was observed by Hoosbeek and Scarascia-Mugnozza (2009).

Afforestation of our experimental fields has increased the litter input in comparison with previous crops, resulting in an increase of NH_4-N in the soil. This increase was significantly smaller under FACE between 2006–2008, probably due to the combination of increased nitrification, microbial activity or plant uptake under FACE. Soil NO_3-N increased after 2005 which may also have been due to the increased turnover of SOM, while larger uptake under FACE may explain lower NO_3-N. The N concentration in *Alnus* and *Betula* leaves was lower under FACE than under ambient CO_2, which means that the demand for more N in order to sustain higher NPP under FACE 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 POP-EuroFACE 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 NPP (labile C) to increase symbiotic N_2-fixation in order to meet the higher N demand under higher NPP. 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 FACE. 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 FACE induced soil C accrual was accompanied by a significant increase in soil N, i.e. FACE did not affect the C/N ratio of the mineral soil. Jastrow et al. (2005) postulated that FACE 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 FACE (Calfapietra et al., 2007). As mentioned before, leaf N content of *Alnus* and *Betula*

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decreased under FACE while the soil C/N ratio was not affected by FACE. From this we infer that at Bangor the major mechanism to sustain increased NPP under FACE is also based on increased NUE. This fits well with the conclusions of (Finzi et al., 2007) (Table 4) 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 FACE.

Acknowledgement. The development of the Bangor FACE site was funded by SRIF. We thank the Forestry Commission Wales and CIRRE for support of the running costs.

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Table 1. Initial soil conditions at the BangorFACE experimental site.

Plot	CO_2 trmt	Clay %	Silt %	Sand %	pH (KCl) mean	pH (KCl) s.e.	NH_4 ($g\,N\,m^{-2}$) mean	NH_4 ($g\,N\,m^{-2}$) s.e.	NO_3 ($g\,N\,m^{-2}$) mean	NO_3 ($g\,N\,m^{-2}$) s.e.	C_{total} ($g\,C\,m^{-2}$) mean	C_{total} ($g\,C\,m^{-2}$) s.e.	N_{total} ($g\,N\,m^{-2}$) mean	N_{total} ($g\,N\,m^{-2}$) s.e.
1	ambient	10	33	57	5.0	0.3	0.09	0.00	0.74	0.07	2753	40	242	6
2	ambient	8	26	67	5.1	0.4	0.10	0.01	0.92	0.09	2569	49	214	8
3	FACE	9	27	64	4.6	0.2	0.10	0.01	0.92	0.12	3068	135	249	10
4	FACE	9	29	62	4.9	0.2	0.07	0.01	0.79	0.19	2898	236	237	27
5	FACE	9	26	65	4.4	0.1	0.08	0.01	0.77	0.44	2171	269	226	32
6	FACE	10	29	61	4.5	0.1	0.10	0.01	0.88	0.23	2786	120	276	12
7	ambient	9	25	66	4.4	0.0	0.08	0.01	0.64	0.06	2808	136	271	9
8	ambient	10	30	60	4.1	0.1	0.08	0.00	0.57	0.05	3191	74	304	8

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Table 2. 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	µg N g^{-1} dw		$\delta^{15}N$		f_n	
		mean	s.e.	mean	s.e.	mean	s.e.
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		
FACE	*Alnus*	60.48	3.41	−0.53	0.13	0.60	0.02
	Betula	56.86	4.04	2.55	0.10		

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Table 3. C and N contents of isolated soil fractions.

Soil C fraction	CO_2 treatment	Species	g C m^{-2}		g N m^{-2}	
			mean	s.e.	mean	s.e.
Coarse POM >250 µm	Ambient CO_2	*Alnus*	788	173	64	15
		Betula	740	124	47	10
		Fagus	727	122	45	11
		Mix	673	93	47	7
	FACE	*Alnus*	993	196	64	29
		Betula	941	42	64	13
		Fagus	761	120	48	4
		Mix	812	135	41	3
Fine POM 53–250 µm	Ambient CO_2	*Alnus*	158	38	13	5
		Betula	131	16	11	2
		Fagus	138	21	12	2
		Mix	149	31	10	2
	FACE	*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 µm	Ambient CO_2	*Alnus*	504	59	32	6
		Betula	610	26	43	5
		Fagus	536	54	47	7
		Mix	536	61	38	4
	FACE	*Alnus*	428	54	27	8
		Betula	485	102	44	17
		Fagus	439	42	30	6
		Mix	442	66	31	10

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Table 4. 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., 2005, 2006; 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 CO_2 – FACE)	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.

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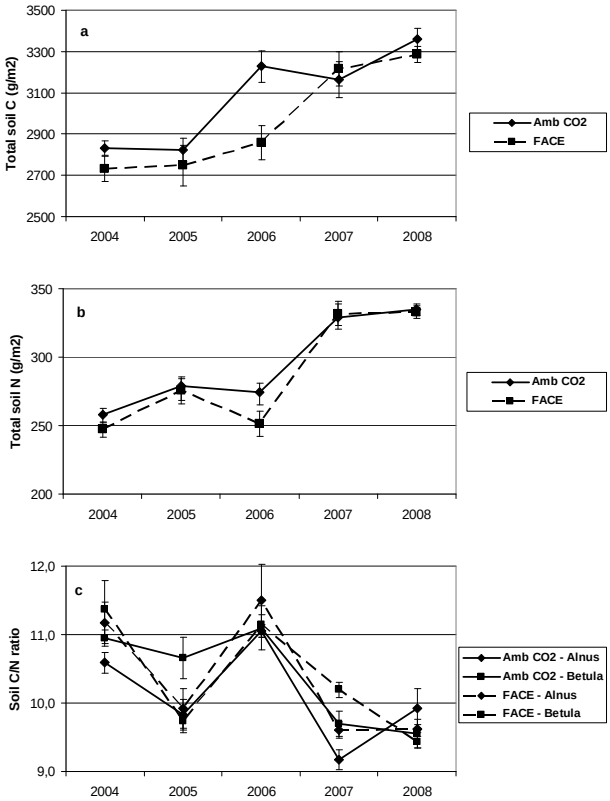

Fig. 1. Change of total soil C **(a)** and N **(b)** and soil C/N ratios **(c)** at 0–10 cm depth. Soil C: significant time effect (P=0.005); no CO_2 treatment effect (P=0.730); no species effect (P=0.628); no significant interactions. Soil N: significant time effect (P=0.001); no CO_2 treatment effect (P=0.767); no species effect (P=0.893); no significant interactions. C/N ratios: significant time effect (P=0.003); no CO_2 treatment effect (P=0.773); no species effect (P=0.058); no significant interactions.

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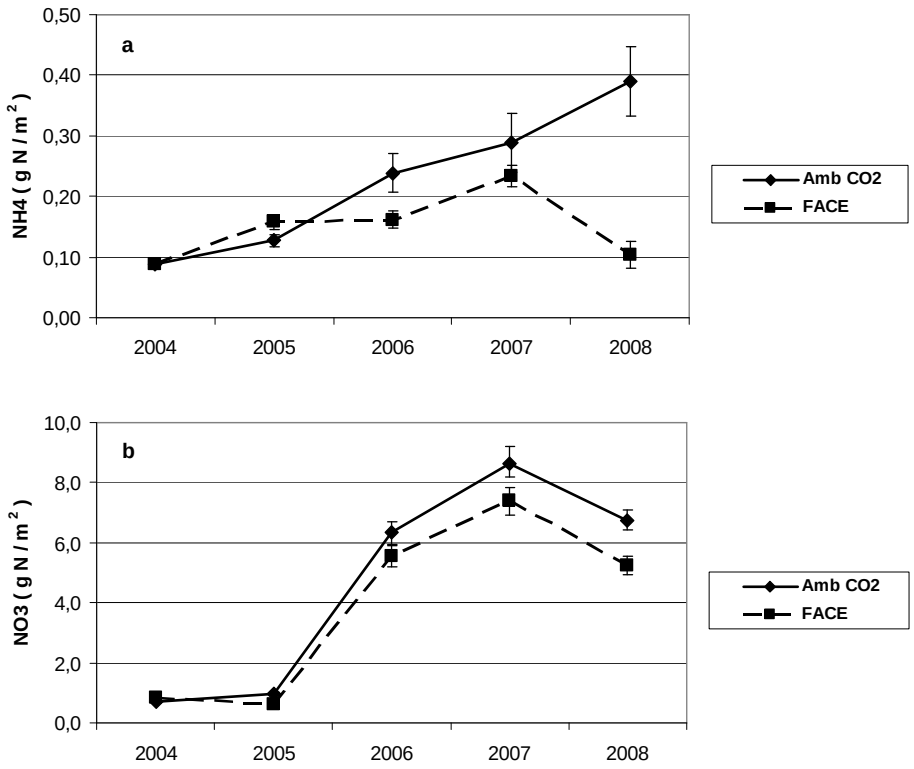

Fig. 2. Change of NH_4-N **(a)** and NO_3-N **(b)** at 0–10 cm depth. NH_4-N: significant FACE effect (P=0.001); no time effect (P=0.092); no species effect (P=0.261); no significant interactions. NO_3-N: significant time effect (P<0.000); no CO_2 treatment effect (P=0.276); no species effect (P=0.319); no significant interactions.

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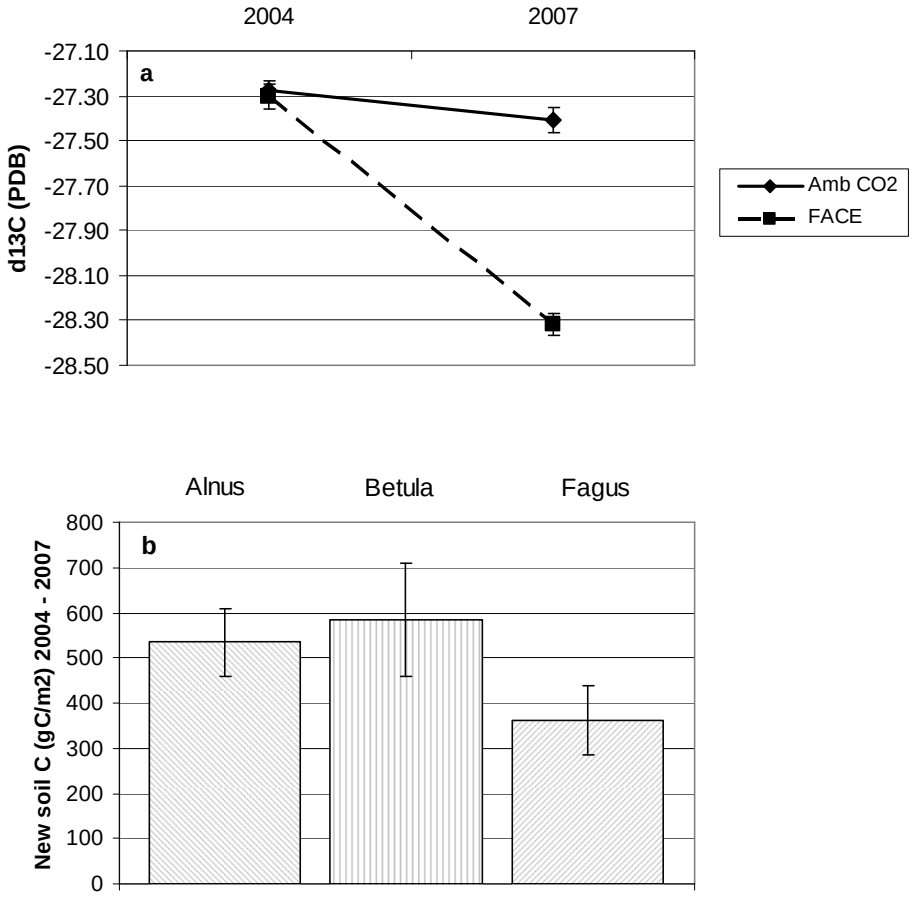

Fig. 3. Change of soil $\delta^{13}C$ **(a)** and new soil C **(b)** in FACE plots at 0–10 cm depth between October of 2004 and 2007.

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