

# Elevated CO<sub>2</sub> increased phosphorous loss from decomposing litter and soil organic matter at two FACE experiments with trees

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**Abstract** Sustained increased productivity of trees growing in elevated CO<sub>2</sub> depends in part on their stoichiometric flexibility, i.e., increasing their nutrient use efficiency, or on increased nutrient uptake from the soil. Phosphorus (P) may be a nutrient as limiting as nitrogen (N) in terrestrial ecosystems and may play a key-process in global terrestrial C storage. For this study archived litter and soil samples of two free air CO<sub>2</sub> enrichment (FACE) experiments were analyzed for C, N and P. *Populus euramericana*, *nigra* and *alba* and *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica* were grown in ambient and elevated CO<sub>2</sub> at respectively the Euro- and BangorFACE experiments. At EuroFACE, aboveground litter accumulated in L, F and H layers, while at BangorFACE almost all aboveground litter was incorporated into the mineral soil due to bioturbation. At EuroFACE, more P was lost from the F and H litter layers due to trees growing in elevated CO<sub>2</sub>, while at BangorFACE more P was lost from the mineral soil. Results of this study imply that trees growing in elevated CO<sub>2</sub> were P limited at both experiments. Therefore, with increasing atmospheric CO<sub>2</sub>, P may play a more pronounced role than previous thought in regulating secondary forest

growth. Moreover, increased atmospheric CO<sub>2</sub> and ample N may allow a larger pool of P to become available for uptake due to, for instance, increased phosphatase activity resulting in increased organic matter turnover and biogenic weathering. Therefore, it may be postulated that under non-N-limited conditions, e.g., during regrowth, under high N deposition or in systems with high N<sub>2</sub>-fixation, increased P availability and uptake may allow P-limited forests to sustain increased growth under increasing atmospheric CO<sub>2</sub>.

**Keywords** Soil phosphorous · Elevated CO<sub>2</sub> · FACE experiment · Litter and soil stoichiometry · Secondary forest growth

## Introduction

Next to nitrogen (N), phosphorous (P) may be limiting to sustained increased tree growth under elevated atmospheric CO<sub>2</sub>. Based on four free air CO<sub>2</sub> enrichment (FACE) experiments in aggrading forests and plantations, Norby et al. (2005) calculated an average CO<sub>2</sub> response of 18 % based on net primary productivity (NPP). The extra N needed to sustain these high rates of NPP under increased atmospheric CO<sub>2</sub> was taken up from the soil at the Duke Forest, Oak Ridge and Aspen FACE experiments (Finzi et al. 2007; Johnson et al. 2004). However, at the EuroFACE

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experiment, the fourth experiment considered by Finzi et al. (2007), the trees increased their N use efficiency (NUE), i.e., their stoichiometric flexibility allowed the extra uptake of C under increased atmospheric CO<sub>2</sub> (Calfapietra et al. 2007; Elser et al. 2010). Also at the BangorFACE experiment increased NUE was observed to be the mechanism for sustained increased NPP under increased atmospheric CO<sub>2</sub> (Hoosbeek et al. 2011). This ability of trees to take up more C with the same amount of N points at tree growth under non-N-limited conditions which raised the question what the role of P was in sustaining increased NPP under elevated CO<sub>2</sub> at the Euro- and BangorFACE experiments.

Changes to the terrestrial N cycle may cause shifts from N- to P-limitation. So far the focus has been mainly on N cycling because there is a tight relationship between the N and C cycles (Gruber and Galloway 2008) and because N is a common limiting factor in temperate ecosystems (Houghton 2007; LeBauer and Treseder 2008). However, P may be a nutrient as limiting as N in terrestrial ecosystems (Elser et al. 2007), and P availability can constrain N<sub>2</sub>-fixation (Niklaus and Körner 2004), which is recognized to be a key-process in global terrestrial C storage under climate change (Van Groenigen et al. 2006; Wang and Houlton 2009). In addition, regional increases of anthropogenic N emissions have caused a change in nutrient composition in various ecosystems (Naples and Fisk 2010), alleviating the primary N limitation to terrestrial ecosystems and inducing a shift towards P limitation (Peñuelas et al. 2012; Vitousek et al. 2010).

Several studies conducted in closed growth and open-top chambers support the potential importance of P for tree growth under elevated CO<sub>2</sub>. Lewis et al. (2010) examined interactive effects of P supply and CO<sub>2</sub> on *Populus deltoides* seedlings growing in controlled growth chambers. They found the growth response at high CO<sub>2</sub> (700 ppm) to be very sensitive to P supply, i.e., as compared to ambient CO<sub>2</sub> (350 ppm), growth under high CO<sub>2</sub> increased by respectively 7 % at low and 80 % at high P supply. Liu et al. (2013) studied the effects of elevated CO<sub>2</sub> and N deposition on N and P concentrations in plant tissues of five tree species growing in model forest ecosystems in open-top chambers in subtropical China. Elevated CO<sub>2</sub> or N addition decreased or had no effect on tissue N:P ratios depending on tree species. However, the combination

of elevated CO<sub>2</sub> and N addition lowered N:P ratios more consistently, which was mostly due to increases of tissue P concentrations through increased P uptake. They postulated that the combination of elevated CO<sub>2</sub> and N deposition more strongly affected P cycling than N cycling in the N-rich but P-limited environment.

Treatment effects on plant nutrient uptake may be evaluated based on changing litter and soil C:N and C:P stoichiometry. For this study archived litter and soil samples of the Euro- and BangorFACE experiments were analysed for C, N and P. As pointed out by Vitousek et al. (2010), nutrient limitation may be inferred from indirect measurements by examining uptake from litter and soil and the evaluation of changing element ratios in litter and soil. In a review by van den Driessche (1974) the relation between foliar analysis and nutrient availability and the environmental factors that may affect this relationship were discussed. Due to the split-plot design with randomly selected ring areas of both FACE experiments, these environmental factors could be assumed to be equal to ambient and elevated CO<sub>2</sub> plots which allows the assumption of a linear logarithmic relation between foliar concentration and availability of respectively N and P. Based on data of 40 nutrient enrichment experiments, Koerselman and Meuleman (1996) found vegetation N:P ratio to be an indication of the relative availability of N and P in the soil. Moreover, they observed the plant N:P ratio to be strongly correlated with the N:P supply ratio where the ratio between N and P indicates whether N or P limits plant growth, not the absolute contents. Therefore, the expected increased uptake of N and P from litter and soil under elevated CO<sub>2</sub> treatment will be evaluated based on changing litter and soil C, N and P contents and on changing C:N and C:P stoichiometry, i.e., relative losses of N and P as compared to C.

Decomposing litter and soil organic matter (SOM) were assumed to be the primary sources for N and P uptake, where N deposition, P weathering, nitrate leaching and leaching of organic forms of N and P were considered to be relatively minor fluxes as compared to N and P uptake. Whether decomposing litter or SOM was the source of N and P depended on the absence and presence of bioturbation at respectively the Euro- and BangorFACE experiments. At EuroFACE, aboveground litter accumulated in L (fresh and partly decomposed), F (fragmented) and

H (humified) layers, while at BangorFACE almost all aboveground litter was incorporated into the mineral soil due to bioturbation.

Both experiments were established on former agricultural soils with, at least initially, relatively high N availability (Hoosbeek et al. 2004, 2011) which allowed trees to increase their NUE as the mechanism for increased NPP under elevated CO<sub>2</sub>. Based on the inference between nutrient uptake and loss from litter and soil and earlier results with respect to C and N cycling (Hoosbeek et al. 2011; Hoosbeek and Scarascia-Mugnozza 2009; Liberloo et al. 2009), the following hypothesis was evaluated: More P was taken up from decomposing litter and/or soil during non-N-limited tree growth in elevated CO<sub>2</sub>.

## Materials and methods

The EuroFACE experiment was established early 1999 on former agricultural fields near Viterbo in central Italy. The annual precipitation is on average 700 mm with dry summers (xeric moisture regime). The loamy soils were classified as Pachic Xerumbrepts (Soil Survey Staff 1992) and initial soil conditions were described by Hoosbeek et al. (2004). Nine hectares were planted with *Populus × euramericana* hardwood cuttings at a density of 0.5 trees m<sup>-2</sup>. Within this plantation three ambient and three elevated CO<sub>2</sub> plots (30 × 30 m<sup>2</sup>) were randomly assigned and planted at a density of 1 tree m<sup>-2</sup> using three different genotypes: *P. × euramericana*, *P. nigra* L. and *P. alba* L. (Scarascia-Mugnozza et al. 2006). Carbon enrichment was achieved by injecting pure CO<sub>2</sub> through laser-drilled holes in tubing mounted on six masts placed in an octagonal pattern with a diameter of about 22 m (Miglietta et al. 2001). The elevated CO<sub>2</sub> concentrations, measured at 1-min intervals, were within 20 % deviation from the pre-set target concentration of 550 ppm for 91 % of the time (1999–2004) (Liberloo et al. 2009). The trees were coppiced after the first three growing seasons (1999–2001). During the second rotation (2002–2004) several new shoots resprouted from the stem and rooting system. At the end of the 6-year experiment (October 2004) forest floor litter samples were collected and separated in L (recent and almost undecomposed litter), F (fragmented and partly decomposed), and H (humified) layer material (Hoosbeek and Scarascia-Mugnozza

2009). Soil samples were collected at 0–10 and 10–20 cm depth.

The BangorFACE experiment was established early 2004 at the Henfaes experimental research area which is located on the coastal plain about 12 km east of Bangor, Wales, UK. The climate is hyperoceanic, with annual rainfall of about 1000 mm. The soil is a fine loamy brown earth over gravel (Rheidol series) and classified as a dystric cambisol according to the FAO system. Initial soil conditions were described in detail by Hoosbeek et al. (2011). Seedlings of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica* were planted at 80 cm spacing in a hexagonal design. Four ambient and four elevated CO<sub>2</sub> plots were randomly located within the plantation in order to form a complete replicated block design. Inside the 8 m diameter plots, species were planted in a pattern that created mixtures containing one, two and three species. Carbon enrichment started in April 2005 and was achieved by injecting pure CO<sub>2</sub> through laser-drilled holes in tubing mounted on eight masts (Miglietta et al. 2001). The elevated CO<sub>2</sub>, measured at 1-min intervals, was within 30 % deviation from the pre-set target concentration of 580 ppm CO<sub>2</sub> for 75–79 % of the time during the photosynthetically active part of 2005–2008. Litter and soil samples were taken randomly from each sub-plot in October of 2008 at 0–10 cm depth.

Archived litter and soil samples of both experiments were analyzed for total organic C and N with an element analyser (Interscience EA 1108). Organic and available P was determined according to Novozamsky et al. (1983). In short, samples were digested by subsequent additions of a selenium–sulphuric acid mixture and peroxide while heated to 330 °C (Gerhardt Kjeldatherm digestion system). After dilution of the digest, P was determined colorimetrically (spectrophotometer Mechatronics Starrcol SC-60-S at 720 nm). Applied to plant or litter samples this method yields total P while application to mineral soil samples yields organic and easily available P (Buurman et al. 1996; Novozamsky et al. 1983).

The statistical models were described in detail by Hoosbeek and Scarascia-Mugnozza (2009) and Hoosbeek et al. (2011) for respectively the Euro- and BangorFACE experiments. In short, both experiments were set up with a replicated split-plot design with respectively 3 (Euro) and 4 (Bangor) blocks with at EuroFACE CO<sub>2</sub> treatment n = 6 (3 ambient and 3 elevated CO<sub>2</sub> plots) and species n = 36 (12 *P. alba*, 12

*P. nigra* and 12 *P. euramericana* subplots) while at BangorFACE CO<sub>2</sub> treatment n = 8 (4 ambient and 4 elevated CO<sub>2</sub> plots) and species n = 24 (8 *B. pendula*, 8 *A. glutinosa* and 8 *F. sylvatica* subplots). The general linear model (IBM SPSS Statistics 22) included CO<sub>2</sub>trmt and species as fixed factors and block as a random factor and all interactions of these factors. Main or interaction effects were considered to be significant when the *P* value of the F-test was <0.05.

## Results

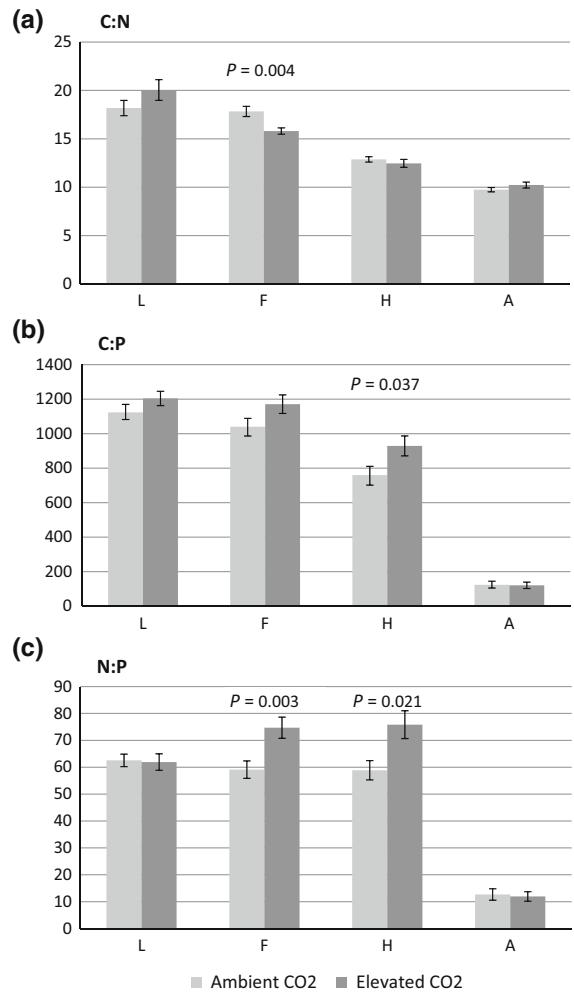
### EuroFACE

After 6 years, above ground litter had accumulated in distinguishable L, F and H litter layers. No signs of bioturbation were observed. Carbon and P content of L litter was larger in the elevated CO<sub>2</sub> plots as compared to the ambient plots (*P* = 0.013 and 0.025; Table 1), while N content was larger as well but not significantly (*P* = 0.089). However, L litter C:N, C:P and N:P ratios were not affected (Fig. 1a–c) which indicates that there was a proportional increase in C, N, and P

**Table 1** CO<sub>2</sub> treatment effect on litter and soil C, N and P contents (n = 36, df = 11) at EuroFACE

	Ambient CO <sub>2</sub>		Elevated CO <sub>2</sub>	
	Mean (g m <sup>-2</sup> )	SE	Mean (g m <sup>-2</sup> )	SE
<b>L litter</b>				
C	176.47	16.87	222.60	17.73
N	9.66	0.84	11.40	1.06
P	0.16	0.01	0.19	0.02
<b>F litter</b>				
C	145.73	15.91	188.30	26.45
N	8.23	0.87	12.14	1.75
P	0.15	0.02	0.16	0.02
<b>H litter</b>				
C	272.46	35.97	338.30	36.50
N	21.68	3.03	27.48	2.80
P	0.37	0.05	0.41	0.06
<b>Soil 0–10 cm</b>				
C	1615.13	33.18	1481.33	57.57
N	165.86	5.31	148.40	7.22
P	17.09	1.77	15.79	1.72

CO<sub>2</sub> treatment effect on L litter C (*P* = 0.013) and P (*P* = 0.025) and H litter N (*P* = 0.035) content



**Fig. 1** a–c CO<sub>2</sub> treatment effect on C:N, C:P and N:P ratios of L, F and H litter layers and A horizon soil at EuroFACE. CO<sub>2</sub> effect on C:N of F litter (*P* = 0.004), C:P of H litter (*P* = 0.037) and N:P of F and H litter (*P* = 0.003 and 0.021). Error bars represent standard error

concentrations under CO<sub>2</sub> treatment which did not affect the stoichiometry of aboveground litter input. Fragmented litter C, N and P contents were not affected by CO<sub>2</sub> treatment, however C:N ratio was smaller (*P* = 0.004) while N:P ratio was larger (*P* = 0.003) under elevated CO<sub>2</sub>. These seemingly contradictory results may be explained by a reduction in variability by taking the ratio of two element contents. For instance, the response ratio of C and N contents are respectively 1.29 and 1.48 but without significant treatment effect due to large SE values, however the C:N values show less variability which

resulted in a significant CO<sub>2</sub> treatment effect. Humified litter N content was larger ( $P = 0.035$ ) in the elevated CO<sub>2</sub> plots, while H litter C and P contents were not affected. However, H litter C:N was not affected, while C:P and N:P were larger under elevated CO<sub>2</sub> ( $P = 0.037$  and  $0.021$ ).

Carbon, N and P contents of the mineral A horizon were larger in the ambient plots, but this difference with the elevated CO<sub>2</sub> plots was inherited from pre-experimental conditions where the average soil C and N contents were non-significantly lower in the FACE plots (Hoosbeek et al. 2004), in fact, soil C, N and P contents were not affected by CO<sub>2</sub> treatment during the experiment, nor was soil stoichiometry affected. Poplar genotype did not affect litter and soil C, N and P stoichiometry.

### BangorFACE

After 4 years, only patches of L litter, i.e., almost undecomposed leaf litter of less than 1 year old, had accumulated. Before fragmentation and further decomposition may have occurred, most litter was incorporated into the mineral top soil by bioturbation, which in this case was clearly due to earth worm activity. Elevated CO<sub>2</sub> treatment did not affect C, N and P percentages of L litter (Table 2), however, N and P percentages in litter originating from *Alnus* were

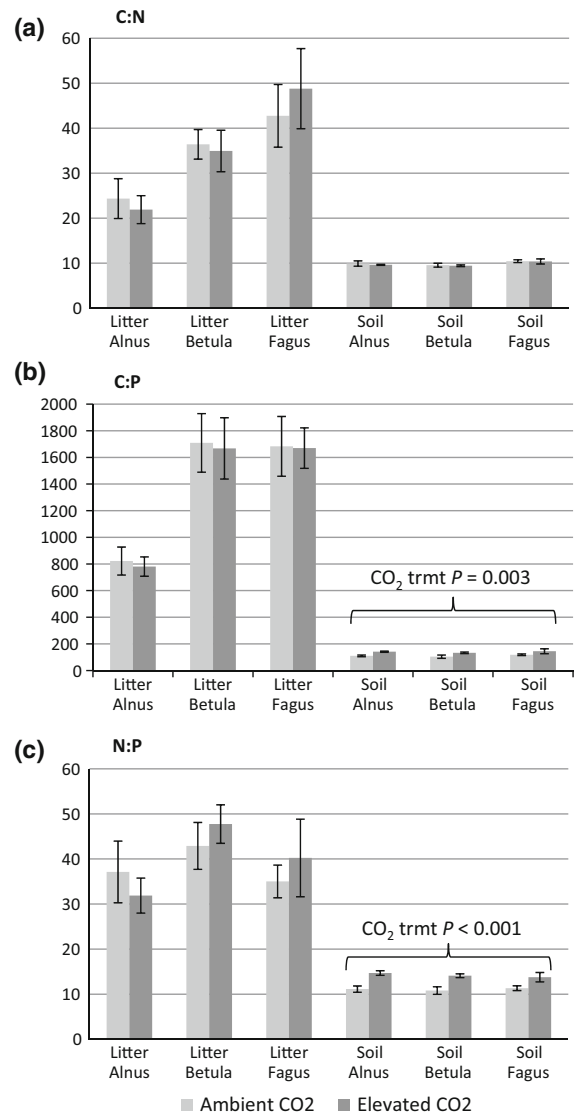
**Table 2** CO<sub>2</sub> treatment and species effects on L leaf litter C, N and P percentages ( $n = 24$ ,  $df = 5$ ) at BangorFACE

	Ambient CO <sub>2</sub>		Elevated CO <sub>2</sub>	
	Mean (%)	SE	Mean (%)	SE
<b>L litter <i>Alnus</i></b>				
C	49.6	0.6	49.4	0.5
N	2.36	0.47	2.50	0.35
P	0.064	0.008	0.066	0.005
<b>L litter <i>Betula</i></b>				
C	51.1	0.4	53.1	0.8
N	1.47	0.18	1.64	0.23
P	0.032	0.005	0.035	0.006
<b>L litter <i>Fagus</i></b>				
C	50.2	2.2	52.7	1.7
N	1.30	0.20	1.22	0.19
P	0.033	0.005	0.033	0.004

Species effect on N ( $P = 0.001$ ) and P ( $P < 0.001$ ) percentages

larger ( $P = 0.001$  and  $<0.001$ ) as compared to litter from *Betula* and *Fagus*. Similarly, litter C:N, C:P and N:P stoichiometry was not affected by CO<sub>2</sub> treatment (Fig. 2a–c), but C:N and C:P ratios of *Alnus* litter were lower ( $P = 0.001$  and  $<0.001$ ) as compared to litter of the other species.

Active bioturbation as observed in the field, e.g., earth worms pulling rolled leaves into soil channels, caused most above ground litter to be decomposed in



**Fig. 2** a–c CO<sub>2</sub> treatment and species effect on C:N, C:P and N:P ratios of litter and soil at BangorFACE. Species effect (*Alnus*) on C:N ( $P = 0.001$ ) and C:P ( $P < 0.001$ ) ratios of litter. CO<sub>2</sub> effect on soil C:P ( $P = 0.003$ ) and soil N:P ( $P < 0.001$ ). Error bars represent standard error

**Table 3** CO<sub>2</sub> treatment and species effects on soil C, N and P contents (g m<sup>-2</sup>) (n = 24, df = 5) at BangorFACE

	Ambient CO <sub>2</sub>		Elevated CO <sub>2</sub>	
	Mean (g m <sup>-2</sup> )	SE	Mean (g m <sup>-2</sup> )	SE
Soil <i>Alnus</i>				
C	3355.8	70.6	3142.9	66.7
N	341.0	16.5	326.8	9.6
P	30.9	2.2	22.3	0.8
Soil <i>Betula</i>				
C	3066.0	393.6	3122.5	261.4
N	317.4	27.1	330.2	22.5
P	29.4	1.7	23.4	1.1
Soil <i>Fagus</i>				
C	3687.5	149.8	3474.5	476.4
N	352.9	5.1	330.6	29.5
P	31.3	1.5	23.9	0.5

CO<sub>2</sub> treatment effect on soil P content ( $P < 0.001$ )

the top mineral soil. While soil C and N contents were not affected by CO<sub>2</sub> treatment, soil P content was lower in the elevated CO<sub>2</sub> plots ( $P < 0.001$ ; Table 3). Species did not affect soil C, N and P contents. Due to lower soil P content, soil C:P and N:P ratios were larger in the elevated CO<sub>2</sub> plots ( $P = 0.003$  and  $< 0.001$ ; Fig. 2b, c).

## Discussion

The Euro- and BangorFACE experiments were initiated on former agricultural soils which allowed the trees to grow initially without N limitation and to show stoichiometric flexibility under increased atmospheric CO<sub>2</sub> during following years, i.e., they increased their N use efficiency. This situation, in which plantations are initiated on former agricultural land, is increasingly common due to the on-going conversion of traditional agriculture to woody biomass production (Liberloo et al. 2010). Also, forest regrowth after land abandonment is usually, at least initially, not N-limited. Other ecosystems that may be P-limited rather than N-limited include primary forests on ferralsols (oxisols) (Nardoto et al. 2014; Nasto et al. 2014; Quesada et al. 2010, 2011) where progressive loss of P has resulted in “depletion-driven P limitation” (Townsend et al. 2008; Vitousek et al. 2010).

At the EuroFACE experiment, total plant C had increased by 21 % after 6 years of elevated CO<sub>2</sub> treatment (Liberloo et al. 2009). Above ground litter accumulated in forest floor litter layers (L + F + H) with respectively 574 (SE 48) and 767 (SE 52) g C m<sup>-2</sup> in ambient and elevated CO<sub>2</sub> plots (Hoosbeek and Scarascia-Mugnozza 2009). A decrease of leaf N content under elevated CO<sub>2</sub> as observed by Cotrufo et al. (2005) did not result in a higher C:N ratio of the L litter under elevated CO<sub>2</sub>. During subsequent fragmentation and decomposition in the F litter layer, the C:N ratio decreased relatively more in elevated CO<sub>2</sub> plots. Carbon content of F litter was not affected by CO<sub>2</sub> treatment, therefore immobilization of N was probably larger in the F layer. Litter C:N of the H layer was not affected, suggesting no additional N-immobilization. At a sub-tropical oak woodland, Hungate et al. (2013) also observed that increased leaf C:N under elevated CO<sub>2</sub> did not change C:N of litter and SOM. They presented increased microbial activity and the increased processing and turnover of soil N in elevated CO<sub>2</sub> plots as a mechanism to decrease soil C:N, i.e., to cancel the increased leaf C:N effect. Similarly, at the Oak Ridge FACE experiment Johnson et al. (2004) observed no CO<sub>2</sub> treatment effect on forest floor nutrient content despite lower leaf litter N concentrations in elevated CO<sub>2</sub>.

With respect to P, this study showed increased F litter N:P and H litter C:P and N:P ratios under elevated CO<sub>2</sub> suggesting extra P depletion which is most likely due to increased uptake by trees. Nutrient uptake from the F and H litter layers is supported by field observations showing coarse roots in the mineral soil branching out upwards into dense networks of fine roots in the H and L litter layers. Moreover, root biomass was observed to be higher under elevated CO<sub>2</sub> (Liberloo et al. 2009). Therefore, based on the inference proposed by Vitousek et al. (2010), apparently more P was taken up from the F and H litter layers by trees growing in elevated CO<sub>2</sub>.

Soil organic C content had increased after 6 years by respectively 301 and 308 g C m<sup>-2</sup> at the 0–10 cm depth increment under ambient and elevated CO<sub>2</sub> due to afforestation (Hoosbeek and Scarascia-Mugnozza 2009). Similarly, soil C:N, C:P and N:P ratios were not affected by CO<sub>2</sub> treatment indicating that the mineral soil was not a source of increased nutrient uptake under elevated CO<sub>2</sub>. However, applying a more elaborate P fractionation method to samples of the

fifth experimental year, Khan et al. (2008) found a positive CO<sub>2</sub> effect on NaOH and HCl-extractable P and suggested that increased fine root and mycorrhizal biomass under elevated CO<sub>2</sub> may have increased mineral weathering and replenishment of available P. This extra replenishment may have compensated increased P uptake resulting in no CO<sub>2</sub> effect on soil organic and available P as was observed in this study.

At the end of the BangorFACE experiment in 2008, aboveground woody biomass was larger in elevated CO<sub>2</sub> than in ambient plots with respectively 6450 and 5497 g m<sup>-2</sup> (Hoosbeek et al. 2011). Despite this increased productivity, elevated CO<sub>2</sub> treatment did not affect C, N and P percentages of litter, nor did it affect litter C:N, C:P and N:P stoichiometry. This lack of CO<sub>2</sub> treatment effect on litter quality may be explained by sufficiently increased nutrient uptake by trees growing in elevated CO<sub>2</sub>.

Litter quality was however affected by tree species, N and P percentages in litter originating from *Alnus* were larger as compared to litter from *Betula* and *Fagus*. Also, litter C:N and C:P ratios of *Alnus* were lower as compared to the other species. This species effect may in part be explained by the symbiotic N<sub>2</sub>-fixing capability of *Alnus*. The extra labile C present in *Alnus* trees growing in increased CO<sub>2</sub> may be available to ‘fuel’ and increase the N<sub>2</sub>-fixation process (Hartwig 1998). Based on δ<sup>15</sup>N measurements, Hoosbeek et al. (2011) found the ratio of N taken up from the soil to N taken up from the atmosphere (N<sub>2</sub>-fixation) not to be affected by elevated CO<sub>2</sub>, which means that N<sub>2</sub>-fixation increased proportionally to increased growth in elevated CO<sub>2</sub>. Still, despite this extra source of N, the extra P needed for increased growth under elevated CO<sub>2</sub> must have been taken up from the soil.

During the 4-year experiment soil C content increased by 530 (SE 53) and 555 (SE 39) g C m<sup>-2</sup> in the ambient and elevated CO<sub>2</sub> plots at 0–10 cm depth increment due to afforestation (Hoosbeek et al. 2011). Despite no CO<sub>2</sub> treatment effect on soil C and N contents and C:N ratios, soil P content was lower and soil C:P and N:P ratios were larger in the elevated CO<sub>2</sub> plots of all species at the end of the experiment. These higher C:P and N:P ratios may be explained by relatively larger plant P uptake from the soil in order to sustain increased growth in elevated CO<sub>2</sub>. Moreover, the increase of N:P shows that more P, as compared to N, was taken up from the elevated CO<sub>2</sub> plots. Working

at the same experiment, Smith (2010) also suggested that P rather than N was the limiting nutrient based on plant-available Olsen-P pools of 153 ± 13 and 83 ± 5 mg P kg<sup>-1</sup> soil in respectively ambient and elevated CO<sub>2</sub> plots (cited in Smith et al. 2013).

Will increasing atmospheric CO<sub>2</sub> alleviate P limited growth in non-N-limited forests?

Depending on the absence or presence of bioturbation at respectively the Euro- and BangorFACE experiments, trees growing in elevated CO<sub>2</sub> presumably took up more P from respectively the F and H litter layers or the mineral soil in order to sustain their increased growth under elevated CO<sub>2</sub>. Instead of looking at nutrient uptake from litter and soil, Liu et al. (2013) studied the “other side” of uptake by examining the change of N:P plant tissue ratios. These N:P ratios decreased due to increased P uptake in response to the combination of elevated CO<sub>2</sub> and N addition. They postulated that this combination of elevated CO<sub>2</sub> and N deposition more strongly affected P cycling than N cycling in the N-rich but P-limited environment. Also at the Euro- and BangorFACE experiments, N limitation was less important because trees were able to increase their NUE in order to sustain their increased growth in elevated CO<sub>2</sub> (Calfapietra et al. 2007; Hoosbeek et al. 2011). Since P loss from litter and SOM may serve as a proxy for nutrient limitation (Koerselman and Meuleman 1996; van den Driessche 1974; Vitousek et al. 2010), the results of this study imply that trees growing in elevated CO<sub>2</sub> were P limited at both experiments. Therefore, under increasing atmospheric CO<sub>2</sub>, P may play a more pronounced role than previously thought in regulating secondary forest growth. Moreover, Liu et al. (2013) suggested that elevated CO<sub>2</sub> and ample N may relieve P limitation due to increased root growth and P uptake. As a result of increased productivity in elevated CO<sub>2</sub>, more labile C will enter litter and soil layers (Hoosbeek et al. 2004) which may speed up the turnover of C and nutrients due to increased root turnover (Lukac et al. 2003; Smith et al. 2013), mycorrhizal hyphal turnover (Godbold et al. 2006) and microbial activity (Johnson et al. 2004; Lagomarsino et al. 2008). An implication of this increased turnover may be the increase of P availability due to biogenically driven mineral weathering (Khan et al. 2008) and/or increased mineralization of organic P which is

controlled by phosphatase enzyme activity (Burns et al. 2013; Sinsabaugh and Follstad Shah 2012). In a meta-analysis, Marklein and Houlton (2012) evaluated the effects of N and P fertilization on phosphatase activity and showed that increased N availability enhanced phosphatase activity. They stressed the coupling between the N and P cycles with increasing N availability resulting in increasing P cycling rates. Therefore, it may be postulated that under non-N-limited conditions as shown in this study, increasing atmospheric CO<sub>2</sub> may alleviate P limited tree growth.

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## References

- Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A (2013) Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol Biochem* 58:216–234
- Buurman P, Van Lagen B, Velthorst EJ (1996) Manual for soil and water analyses. Backhuys Publishers, Leiden
- Calfapietra C, De Angelis P, Gielen B, Lukac M, Moscatelli MC, Avino G, Lagomarsino A, Polle A, Ceulemans R, Scarascia-Mugnozza G, Hoosbeek MR, Cotrufo MF (2007) Increased nitrogen-use efficiency of a short-rotation poplar plantation in elevated CO<sub>2</sub> concentration. *Tree Physiol* 27:1153–1163
- Cotrufo MF, De Angelis P, Polle A (2005) Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO<sub>2</sub> enrichment (POPFACE). *Glob Change Biol* 11:971–982
- Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10(12):1135–1142
- Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, Enquist BJ (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *N Phytol* 186(3):593–608
- Finzi AC, Norby RJ, Calfapietra C, Gallet Budynek A, Gielen B, Holmes WE, Hoosbeek MR, Iversen CM, Jackson RB, Kubiske ME, Ledford J, Liberloo M, Oren R, Polle A, Pritchard S, Zak DR, Schlesinger WH, Ceulemans R (2007) Increases in nitrogen uptake rather than nitrogen-use efficiency support higher rates of temperate forest productivity under elevated CO<sub>2</sub>. *Proc Natl Acad Sci USA* 104(35):14014–14019
- Godbold D, Hoosbeek M, Lukac M, Cotrufo MF, Janssens I, Ceulemans R, Polle A, Velthorst E, Scarascia-Mugnozza G, De Angelis P, Miglietta F, Peressotti A (2006) Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant Soil* 281(1–2):15–24
- Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. *Nature* 451:293–296
- Hartwig UA (1998) The regulation of symbiotic N<sub>2</sub> fixation: a conceptual model of N feedback from the ecosystem to the gene expression level. *Perspect Plant Ecol Evol Syst* 1(1):92–120
- Hoosbeek MR, Scarascia-Mugnozza GE (2009) Increased litter build up and soil organic matter stabilization in a poplar plantation after 6 years of atmospheric CO<sub>2</sub> enrichment (FACE): final results of POP-EuroFACE compared to other forest FACE experiments. *Ecosystems* 12:220–239
- Hoosbeek MR, Lukac M, Van Dam D, Godbold DL, Velthorst EJ, Biondi FA, Peressotti A, Cotrufo MF, De Angelis P, Scarascia-Mugnozza G (2004) More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): cause of increased priming effect? *Glob Biogeochem Cycles* 18:GB1040. doi:10.1029/2003GB002127
- Hoosbeek MR, Lukac M, Velthorst EJ, Smith AR, Godbold DL (2011) Free atmospheric CO<sub>2</sub> enrichment increased above ground biomass but did not affect symbiotic N<sub>2</sub>-fixation and soil carbon dynamics in a mixed deciduous stand in Wales. *Biogeosciences* 8:353–364
- Houghton RA (2007) Balancing the global carbon budget. *Annu Rev Earth Planet Sci* 35(1):313–347
- Hungate BA, Dijkstra P, Wu Z, Duval BD, Day FP, Johnson DW, Megonigal JP, Brown ALP, Garland JL (2013) Cumulative response of ecosystem carbon and nitrogen stocks to chronic CO<sub>2</sub> exposure in a subtropical oak woodland. *N Phytol* 200(3):753–766
- Johnson DW, Cheng W, Joslin JD, Norby RJ, Edwards NT, Todd DE Jr (2004) Effects of elevated CO<sub>2</sub> on nutrient cycling in a sweetgum plantation. *Biogeochemistry* 69(3):379–403
- Khan FN, Lukac M, Turner G, Godbold DL (2008) Elevated atmospheric CO<sub>2</sub> changes phosphorus fractions in soils under a short rotation poplar plantation (EuroFACE). *Soil Biol Biochem* 40(7):1716–1723
- Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *J Appl Ecol* 33(6):1441–1450
- Lagomarsino A, Moscatelli MC, Hoosbeek MR, De Angelis P, Grego S (2008) Assessment of soil nitrogen and phosphorus availability under elevated CO<sub>2</sub> and N-fertilization in a short rotation poplar plantation. *Plant Soil* 308:131–147



- LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89(2):371–379
- Lewis JD, Ward JK, Tissue DT (2010) Phosphorus supply drives nonlinear responses of cottonwood (*Populus deltoides*) to increases in CO<sub>2</sub> concentration from glacial to future concentrations. *N Phytol* 187(2):438–448
- Liberloo M, Lukac M, Calfapietra C, Hoosbeek MR, Gielen B, Miglietta F, Scarascia-Mugnozza GE, Ceulemans R (2009) Coppicing shifts CO<sub>2</sub> stimulation of poplar productivity to above-ground pools: a synthesis of leaf to stand level results from the POP/EUROFACE experiment. *N Phytol* 182(2):331–346
- Liberloo M, Luysaert S, Bellassen V, Njakou Djomo S, Lukac M, Calfapietra C, Janssens IA, Hoosbeek MR, Viovy N, Churkina G, Scarascia-Mugnozza G, Ceulemans R (2010) Bio-energy retains its mitigation potential under elevated CO<sub>2</sub>. *PLoS One* 5(7):e11648
- Liu J, Huang W, Zhou G, Zhang D, Liu S, Li Y (2013) Nitrogen to phosphorus ratios of tree species in response to elevated carbon dioxide and nitrogen addition in subtropical forests. *Glob Change Biol* 19(1):208–216
- Lukac M, Calfapietra C, Godbold DL (2003) Production, turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated CO<sub>2</sub> (POPFACE). *Glob Change Biol* 9:838–848
- Marklein AR, Houlton BZ (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *N Phytol* 193(3):696–704
- Miglietta F, Peressotti A, Vaccari FP, Zaldei A, De Angelis P, Scarascia-Mugnozza G (2001) Free-air CO<sub>2</sub> enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *N Phytol* 150:465–476
- Naples B, Fisk M (2010) Belowground insights into nutrient limitation in northern hardwood forests. *Biogeochemistry* 97(2):109–121
- Nardoto GB, Quesada CA, Patiño S, Saiz G, Baker TR, Schwarz M, Schrodt F, Feldpausch TR, Domingues TF, Marimon BS, Marimon Junior B-H, Vieira ICG, Silveira M, Bird MI, Phillips OL, Lloyd J, Martinelli LA (2014) Basin-wide variations in Amazon forest nitrogen-cycling characteristics as inferred from plant and soil 15N:14N measurements. *Plant Ecol Divers* 7(1–2):173–187
- Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC (2014) Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. *Ecol Lett* 17(10):1282–1289
- Niklaus PA, Körner C (2004) Synthesis of a six-year study of calcareous grassland responses to in situ CO<sub>2</sub> enrichment. *Ecol Monogr* 74(3):491–511
- 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 G, Schlesinger WH, Oren R (2005) Forest response to elevated CO<sub>2</sub> is conserved across a broad range of productivity. *Proc Natl Acad Sci USA* 102(50):18052–18056
- Novozamsky I, Houba VJG, Van Eck R, Van Vark W (1983) A novel digestion technique for multi-element plant analysis. *Commun Soil Sci Plant Anal* 14(3):239–248
- Peñuelas J, Sardans J, Rivas-ubach A, Janssens IA (2012) The human-induced imbalance between C, N and P in Earth's life system. *Glob Change Biol* 18(1):3–6
- Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmerler J, Santos AJB, Hodnett MG, Herrera R, Luizão FJ, Arneth A, Lloyd G, Dezzio N, Hilke I, Kuhlmann I, Raessler M, Brand WA, Geilmann H, Moraes Filho JO, Carvalho FP, Araujo Filho RN, Chaves JE, Cruz Junior OF, Pimentel TP, Paiva R (2010) Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* 7(5):1515–1541
- Quesada CA, Lloyd J, Anderson LO, Fyllas NM, Schwarz M, Czimczik CI (2011) Soils of Amazonia with particular reference to the RAINFOR sites. *Biogeosciences* 8(6):1415–1440
- Scarascia-Mugnozza GE, Calfapietra C, Ceulemans R, Gielen B, Cotrufo MF, De Angelis P, Godbold DL, Hoosbeek MR, Kull O, Lukac M, Marek M, Miglietta F, Polle A, Raines C, Sabatti M, Anselmi N, Taylor G (2006) Responses to elevated [CO<sub>2</sub>] of a short rotation, multispecies poplar plantation: the POPFACE/EUROFACE experiment. In: Nösberger J, Long SP, Norby RJ, Stitt M, Hendrey GR, Blum H (eds) *Managed ecosystems and CO<sub>2</sub>. Ecological studies*. Springer, Berlin, pp 173–195
- Sinsabaugh RL, Follstad Shah JJ (2012) Ecoenzymatic stoichiometry and ecological theory. *Annu Rev Ecol Evol Syst* 43(1):313–343
- Smith AR (2010) The effect of atmospheric CO<sub>2</sub> enrichment on biogeochemical cycling of a temperate forest ecosystem. School of Environment, Natural Resources and Geography, Bangor University, Bangor
- Smith AR, Lukac M, Bambrick M, Miglietta F, Godbold DL (2013) Tree species diversity interacts with elevated CO<sub>2</sub> to induce a greater root system response. *Glob Change Biol* 19(1):217–228
- Soil Survey Staff (1992) *Keys to soil taxonomy*, sixth edition, 1994. Soil Conservation Service, USDA, Washington, DC
- Townsend AR, Asner GP, Cleveland CC (2008) The biogeochemical heterogeneity of tropical forests. *Trends Ecol Evol* 23(8):424–431
- van den Driessche R (1974) Prediction of mineral nutrient status of trees by foliar analysis. *Bot Rev* 40(3):347–394
- Van Groenigen KJ, Six J, Hungate BA, De Graaff MA, Van Breemen N, Van Kessel C (2006) Element interactions limit soil carbon storage. *Proc Natl Acad Sci USA* 103(17):6571–6574
- Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecol Appl* 20(1):5–15
- Wang Y-P, Houlton BZ (2009) Nitrogen constraints on terrestrial carbon uptake: implications for the global carbon-climate feedback. *Geophys Res Lett* 36(24):L24403