

## TREE EXPOSURE TO ELEVATED CO<sub>2</sub> INCREASES AVAILABILITY OF SOIL PHOSPHORUS

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### Abstract

Future high levels of atmospheric carbon dioxide will increase biomass production of terrestrial plants, however depletion of soil mineral nutrients may act as a negative feedback to increased growth. To test this, an ecosystem phosphorus budget was calculated in poplar grown under field conditions at ambient and elevated atmospheric CO<sub>2</sub> for 5 years. The pools of total, plant available, weatherable and organic P were estimated, as well as the P storage in tree biomass components. While as a non-significant increase in amount of P taken up by the trees we observed, plant available P pools in the soil increased significantly. An increase in all soil P extractions was seen, with the greatest increase in an acid soluble P fraction which is considered to be the weatherable fraction. The formation of this P fraction may be biogenically driven and this additional P probably originates from weathering of occluded mineral pools.

### Introduction

The response of plants to increased atmospheric carbon often includes an increase in the rate of assimilation (Ainsworth & Long, 2006). As a consequence of this increase, elevated atmospheric CO<sub>2</sub> has been shown to increase growth of trees. In a number of Free Air Carbon dioxide Enrichment (FACE) sites using trees, a 23 % increase in growth was shown due to enrichment of 200 ppm above ambient. This increase is consistent across a range of net primary productivity (Norby *et al.*, 2005). However, if faster and more vigorous plant growth is to be sustained, either a sufficient nutrient supply must be available (Oren *et al.*, 2001) or nutrient use efficiency must increase. Phosphorus is one of the key elements often considered to limit productivity in terrestrial ecosystems. In a range of forests, an increase in the N:P ratio has been suggested to lead to a decline in growth and stagnation (Wardle *et al.*, 2004). A lack of sufficient P supply has also been shown to cause down-regulation of stimulating effects of elevated carbon dioxide in legumes (Campbell & Sage, 2006).

Phosphorus in soils occurs in pools of varying availability to plants, and the transformation of P between these pools is driven by a complex web of chemical and biological processes (Oldham, 2003; Sanyal & Datta, 1991). Phosphorus is found both in inorganic mineral forms, and in organic forms resulting from plant biomass turnover. Both forms are found in a continuum of compounds, ranging from readily plant available P dissolved in the soil solution to poorly available P held in stable compounds (Larsen, 1967). Available P pool is thought to be replenished by the turnover of organic P and by the weathering of minerals in the poorly available pool (Tiessen & Stewart, 1985). In forest soils, organically bound P generally accounts for 20-80% of total P (Newbery *et al.*, 1997; Tiessen *et al.*, 1994).

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Both plants (Kelly *et al.*, 1998) and associated mycorrhizal fungi (Jongmans *et al.*, 1997) have been suggested to weather minerals in soils. A relationship between net primary productivity and rates of weathering has been suggested, but the rates may be modified by a number of factors (Kelly *et al.*, 1998). Elevated CO<sub>2</sub> increases net primary productivity (Norby *et al.*, 2005), resulting in larger inputs of organic matter into the soil, thus potentially increasing the organic P pool in the soil, but also changing relative abundance of mineral P fractions by affecting mineral weathering (Williams *et al.*, 2003).

Element ratios in leaves and soil extracts have been utilized in an attempt to determine the source of elements mobilized by mineral weathering. Blum *et al.*, (2002) used both element ratios (Sr:Ca) and isotopic Sr ratios (<sup>87</sup>Sr/<sup>86</sup>Sr) in needles to determine the source of Ca being taken up by red spruce (*Picea rubens*). Based on these ratios, they suggested that apatite was being weathered by ectomycorrhizal fungi and used as a Ca source. Apatite is used by ectomycorrhizal fungi also as a source of P (Wallander, 2000). However, the use of Sr/Ca ratios in leaves and needles to predict the sources of elements from soils assumes that there is no fractionation in roots and boles during transport from the soil solution to the leaves. This has, however, been shown not to be true (Bailey *et al.*, 1996). The elemental ratios also change due to more availability of P. As P availability is increased due to elevated level of CO<sub>2</sub> (Liberloo *et al.*, 2006) so it can reduce the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in plants (Naheed *et al.*, 2008) which otherwise be harmful for plants (Naheed *et al.*, 2007), thus enhancing the growth and yield of plants. To assess a greater range of element ratios, Bullen & Bailey (2005) used a multi-tracer approach and generated a number of element ratios.

We have grown three *Populus* species in ambient and elevated CO<sub>2</sub> for 5 years and observed a significant positive effect of elevated CO<sub>2</sub> on above- and belowground tree biomass production (Gielen *et al.*, 2005; Liberloo *et al.*, 2006) and also on mycorrhizal colonisation of poplar roots (Lukac *et al.*, 2003). Unless phosphorus use efficiency was greatly increased in elevated CO<sub>2</sub> treatment, *Populus* trees should have taken up larger amounts of P from the soil to sustain observed increased growth rates. To test this, we hypothesized that greater biomass production in elevated CO<sub>2</sub> treatment would result in greater P uptake and hence in diminished P availability in the soil. Further, by comparing the elemental ratios in soil extracts, we have attempted to pinpoint the mineral source of soil P.

## Materials and Methods

**Site description:** Since planting as stem cuttings in 1999, three species of poplar; *Populus alba*, *P. nigra* and *P. x euramericana* were grown at ambient and elevated atmospheric CO<sub>2</sub> concentration [550ppm] at the EuroFACE experimental facility (Viterbo, Central Italy). The site consisted of three ambient and three elevated CO<sub>2</sub> experimental plots. Each plot was split into 2 halves by a 1m deep barrier and each half was further divided into 3 segments. Each segment was planted with a single *Populus* species. One half of each plot received high nitrogen treatment, while the other was left as a control. All samples discussed in this work were taken from the low nitrogen half of each plot, high nitrogen treatment will therefore not be commented upon in this manuscript. Carbon dioxide enrichment was maintained by the FACE (Free Air Carbon dioxide Enrichment) technique during photosynthetically active part of the year only (Miglietta *et al.*, 2001) and by 2003 had resulted in a significant stimulation of biomass production. During the first three years the trees grew to a height of 15 m. In early 2002

they were coppiced, after which by 2004, when soil samples were taken, the poplars had re-grown to a height of 10 m. Average annual dry woody biomass production had increased from 20.9 to 25.8 Mg ha<sup>-1</sup> in ambient conditions, from 28.0 to 31.0 Mg ha<sup>-1</sup> under elevated CO<sub>2</sub> (Liberloo *et al.*, 2006). The CO<sub>2</sub> concentration within the FACE plots was 544 ± 48 ppm, 532 ± 83 ppm and 554 ± 95 ppm during the first, second and third year before coppice (1999-2001), 554 ± 16 ppm during the first year after coppice (2002) and 535.9 ± 20 ppm during the second year after coppice (2003) (*F. Miglietta*, CNR-IATA, Florence, Italy, unpublished data).

An analysis of the site soil conditions was carried out before the onset of the experiment to account for initial differences between experimental plots and no significant difference in total C, total N and soil pH was observed between the plots allocated to CO<sub>2</sub> treatments. Soil P content was not measured at this point as we did not expect this to differ, given the similarity of other soil characteristics among the plots. The soil, an Inceptisol, was classified as Pachic Xerumbrept-silt loam except for plot 5, which was an Ochrept. The soil profile was more than 1 metre deep, originating from a geological substrate derived from sedimentary material of volcanic origin. The average stone content (fraction >2mm) of the top layer (0-20 cm) was 1.93 % and the soil had a pH of 5.04. The average bulk density was 1.34 g cm<sup>-3</sup> with a C/N ratio of 8.0 (Hoosbeek *et al.*, 2004).

**Soil sampling and analysis:** Soil samples were taken to a depth of 60 cm from soils under each *Populus* species in the 3 ambient and 3 elevated CO<sub>2</sub> plots (FACE) in September 2003. Two soil cores (8 cm in diameter) were taken within each species area to a depth of 60 cm and then divided to depths of 0-10, 10-20, 30-40 and 50-60 cm. Samples from the same depth were then pooled to form one sample per depth per species area. Samples from each depth were analyzed separately and then summed up to obtain total P in the soil to a depth of 60 cm. Values for 20-30 and 40-50cm layers were interpolated from the values of the soil layers immediately above and below. Soil samples were oven dried at 70°C for 72 hours, after which they were ground and passed through a 2mm sieve and stored in polythene bags for further analyses.

Inorganic P fractions in soils were analyzed using a modified Hedley fractionation (Hedley & Stewart, 1982). In detail, we measured the availability of soil P using a sequential extraction method with the following four extraction solutions: H<sub>2</sub>O, 0.1M NaOH and 1M HCl all at 20°C and finally HNO<sub>3</sub> at 130°C in Teflon digestion vessels. The fraction extracted in water encompasses the pool from which plants normally draw their P supply (Amer *et al.*, 1955; Bowman & Cole, 1978). The extraction with 0.1 M NaOH solution partially dissolves iron and aluminium phosphates (Chang & Jackson, 1957), desorbs P from the surfaces of sesquioxides (McLaughlin, 1978; Parfitt, 1978) and probably extracts some organic P. The sum of H<sub>2</sub>O and NaOH fractions is thought to constitute the plant available fraction. Subsequent to alkaline extraction, 1M HCl extracts acid soluble P which forms the weatherable P fraction. This includes P from calcium phosphate and some P occluded within sesquioxides and released on partial dissolution of these oxides. Finally, concentrated HNO<sub>3</sub> has been suggested to dissolve plagioclase and K-feldspar (Blum *et al.*, 2002), giving the total soil P. Potentially plant available P was determined in the H<sub>2</sub>O and NaOH extracts as Mo reactive P colorimetrically with Ames reagent (Bochner & Ames, 1982). The total P in each fraction was then estimated by Induction Coupled Plasma spectrometry (OES-ICP) from all 0-10cm depth extracts. In the water, HCl and HNO<sub>3</sub> extracts Ba, Ca, K, Mg, Na and Sr were also measured by ICP.

The total soil P content was also measured by an independent analysis done by the Institut für Düngermittel und Saatgut, LUFA, Germany, where HNO<sub>3</sub>/HF pressure microwave digestion and subsequent ICP measurement were applied.

Organic P was determined by comparing the P concentration in ignited and non-ignited soils (Walker & Adams, 1958). The samples were ignited at 450 °C and extracted in 0.1 M NaOH.

**Plant materials:** To estimate phosphorus in tree biomass and its potential return to the soil, P concentration in leaves, stems, coarse and fine roots was measured. Leaves (youngest fully expanded leaf), coarse (>2mm) and fine (< 2mm) roots, and wood (mid-stem disks) samples were collected in 2003 and 2005 from all 3 species. In 2003, in each plot 10 of the youngest fully expanded leaves were collected from 3-4 trees within reach of the canopy access tower to make a composite sample. Root samples were taken separately from above mentioned soil samples, using an 8 cm diameter soil corer to depths of 40 cm, since only negligible proportion of root biomass was found below this depth (Lukac *et al.*, 2003). For each species in each plot and treatment, 2 cores per segment were taken to form a composite sample. The cores were separated into soil depths of 0-20 and 20-40 cm, and the roots were removed from the cores and washed. Root samples were first air dried and subsequently oven dried at 60°C for 72 hrs. The wood samples for *Populus alba* and *Populus x euramericana* were collected in 2003 and those for *Populus nigra* in 2005. All materials were digested in HNO<sub>3</sub> in Teflon vessels at 130°C. In the extracts, Mo reactive P was determined colorimetrically using Ames reagent (Bochner & Ames, 1982).

**P budget:** The total P stock in the soils was determined using the P concentration values obtained from exhaustive digestion and ICP/AES analysis and the soil bulk density (Hoosbeek *et al.*, 2004). The plant available pool was calculated from the Mo-reactive P concentrations of the water and NaOH extracts and the weatherable pool from the HCl extracts. The P stored in tree biomass was calculated using the P values for each plant part and the dry biomass production of stems, roots, and leaf litter fall values (Gielen *et al.*, 2005; Liberloo *et al.*, 2006; Lukac *et al.*, 2003). These values were then used to estimate tree and soil P pools.

All data were analyzed by two-way ANOVA (*Sigma Stat 3.0.1; SPSS Inc.*) with CO<sub>2</sub> treatment and species as independent factors, all differences are reported as significant at the 0.05 level.

## Results

The total P pool in the rooting zone was estimated using the HF/HNO<sub>3</sub> extraction method, showing that there was strong spatial variation in P pools across the experimental site (from 221.9 to 462.5 P m<sup>-2</sup> in 0-60cm), however there was no CO<sub>2</sub> effect on total P pool (p=0.266). Using sequential extraction, only ca. 35 % of this total P pool was found to be easily plant available, potentially weatherable or in the organic fraction (Table 1). The plant available P pool was estimated using the H<sub>2</sub>O and NaOH extraction. Under elevated CO<sub>2</sub>, plant available P pool increased by 48% (p=0.006), primarily due to a higher NaOH extractable P pool (p=0.007). The water extractable fraction was not affected by elevated CO<sub>2</sub> and nor was the organic P pool (Table 1). The greatest increase was seen in the HCl extractable fraction, which is considered to be the weatherable pool. The increase in HCl extractable pool contributed 68% of the total P increase in soils, and was observed in all FACE plots.

**Table 1. Changes in soil phosphorus pools in 0-60 cm depth [g P m<sup>-2</sup>, ± SE] of differing plant availability based on sequential extraction from soils under 3 species of *Populus* exposed for 5 years to elevated or ambient atmospheric CO<sub>2</sub> levels, N.S. – not significant.**

Extraction fraction	Species	Ambient [g P m <sup>-2</sup> ]	FACE [g P m <sup>-2</sup> ]	Mean FACE effect [%]
OP	<i>P. alba</i>	18.1 (±5.58)	23.0 (±0.15)	N.S.
	<i>P. nigra</i>	19.3 (±4.42)	23.8 (±1.67)	
	<i>P. x euramericana</i>	17.6 (±7.19)	27.6 (±3.32)	
H <sub>2</sub> O	<i>P. alba</i>	6.5 (±2.3)	6.5 (±1.8)	N.S.
	<i>P. nigra</i>	7.1 (±1.5)	7.1 (±1.7)	
	<i>P. x euramericana</i>	6.9 (±1.7)	7.7 (±1.1)	
NaOH	<i>P. alba</i>	24.5 (±5.6)	35.6 (±4.2)	+60% (P=0.007)
	<i>P. nigra</i>	26.3 (±4.1)	38.6 (±2.7)	
	<i>P. x euramericana</i>	20.9 (±3.4)	41.2 (±5.5)	
HCl	<i>P. alba</i>	34.6 (±3.4)	79.3 (±36.9)	+112% (P=0.017)
	<i>P. nigra</i>	45.1 (±7.1)	86.9 (±34.7)	
	<i>P. x euramericana</i>	42.8 (±2.2)	93.6 (±20.3)	
HF/HNO <sub>3</sub>	pooled	268.7 (±33.0)	360.9 (±63.3)	N.S.

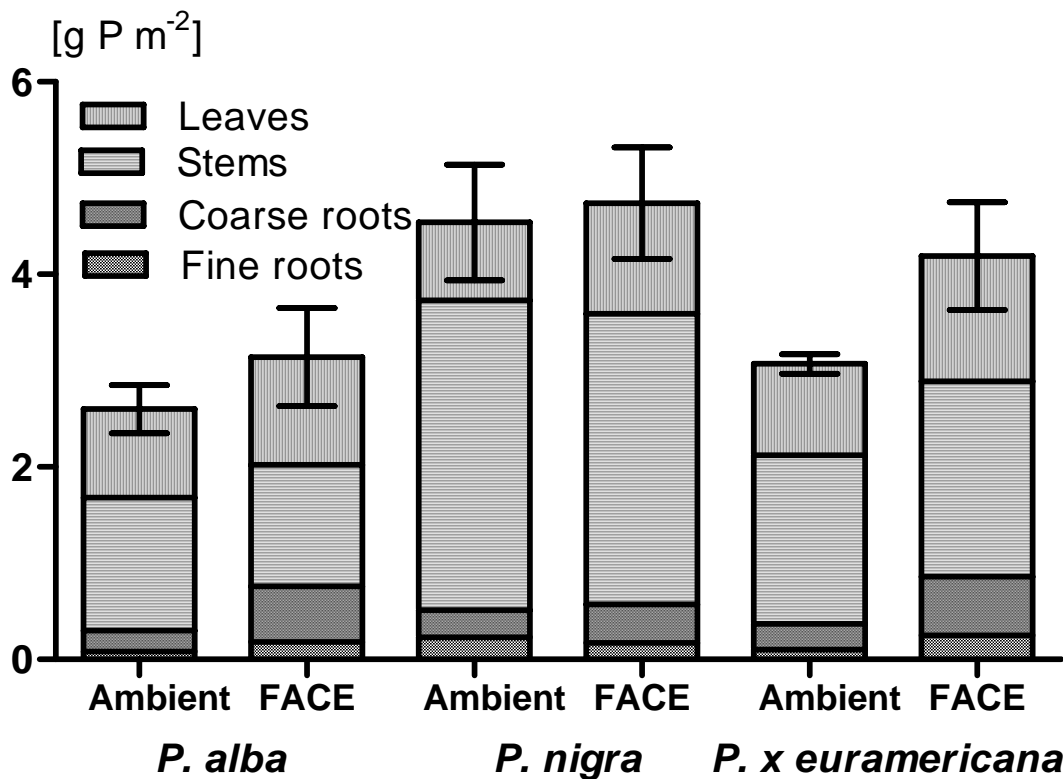


Fig. 1. Phosphorus pools in living biomass of 3 species of *Populus* [g P m<sup>-2</sup>, ± SE of the summed pool].

The P pool in total living biomass constituting of stems, leaves and fine and coarse roots was non-significantly increased under elevated CO<sub>2</sub> by 21% on average across species (p=0.131, Fig. 1). When looking at separate biomass pools, the amount of P held in roots increased significantly on average across species (p=0.010), while *P. x euramericana* was different significantly from *P. alba* (p=0.003) and marginally different from *P. nigra* (p=0.054). However, since the amount of P stored in the biomass was only ca. 2-3% of the P stored in the soil, the response of the total P budget in this ecosystem to FACE is dominated by the response of the soil P.

**Table 2. Calcium to strontium molar ratios in leaves and roots of 3 species of *Populus* exposed for 5 years to elevated or ambient atmospheric CO<sub>2</sub> levels.**

	<i>P. alba</i>		<i>P. nigra</i>		<i>P. x euramericana</i>	
	Ambient	FACE	Ambient	FACE	Ambient	FACE
Leaves	300 ± 20	281 ± 34	263 ± 13	247 ± 38	246 ± 12	247 ± 32
Roots	51 ± 2.5	52 ± 3.3	50 ± 4.6	49 ± 2.6	44 ± 2.8	53 ± 3.9

**Table 3. Ratios of selected elements in the HCl extracts (m mol/m mol, ± SE) and their content in the top 10 cm of the soil measured by ICP (mg kg<sup>-1</sup> ± SE), N.S. – not significant.**

Ratio, element	Ambient	FACE	FACE effect
Ba:Sr	3.86 (±0.83)	5.53 (±0.53)	+43% (p=0.02)
K:Sr	482 (±47)	494 (±46)	N.S.
Ca:Sr	89.2 (±11.0)	104.0 (±14.9)	N.S.
Ca:P	24.5 (±2.3)	19.7 (±9.0)	N.S.
Ca:(Ca+Na)	0.93 (±0.02)	0.80 (±0.05)	N.S.
Mg:K	0.09 (±0.01)	0.10 (±0.01)	N.S.
P	2.84 (±0.43)	6.54 (±2.2)	+130% (p=0.035)
Sr	0.74 (±0.03)	0.80 (±0.06)	N.S.
Ca	65.2 (±6.4)	81.5 (±6.7)	+25% (p=0.02)
Na	4.37 (±1.46)	8.67 (±2.04)	N.S.
Mg	34.2 (±3.4)	41.0 (±7.1)	N.S.
Ba	2.87 (±0.66)	4.47 (±0.77)	+56% (p=0.027)
K	352 (±24)	393 (±43)	N.S.
Al	146 (±28)	222 (±25)	+51% (p= 0.007)

The Ca:Sr molar ratios in leaves did not significantly differ between the ambient and FACE treatments nor between the *Populus* species (Table 2). Similarly, there were no significant differences in the Ca:Sr molar ratios in roots between treatment and species, however the values in roots were 5-6 times lower than those in the leaves.

The elemental composition and ratios in the HCl and HNO<sub>3</sub> extracts from the top soil (0-10 cm) were analyzed by ICP-EMS extracts (Table 3). Under FACE, only the Ba:Sr ratio increased significantly, (p=0.020). The Ca:Sr ratio increased slightly (N.S.), while the other calculated ratios did not change. If the concentrations are examined, the molar concentration of P was increased by 130% under FACE (p=0.035), that of Ba by 56% (p=0.027), Al by 51% (p=0.007) and that of Ca by 25% (p=0.020, Table 3). No significant changes were found in the other elements. In the water and HNO<sub>3</sub> extractions the Ca:Sr molar ratios ranged between 75 to 110 and 40-60, respectively, and did not differ among treatments and species.

## Discussion

Molybdenum-reactive P is commonly used to estimate plant available P in fractionation studies (Haygarth *et al.*, 1995). The method does not measure the total P in solution, but primarily the ortho-P fractions and some labile organic P which is hydrolyzed in the acidic Ames reagent (Kuo, 1996). In our extractions, Mo-reactive P formed only 38% in ambient and 44% in FACE of total P in solution (Khan *et al.*, 2008), while the organic bound P pool constitutes only 6% of the total P pool. However, some of

the measured organic P may be held in organic matter pools with very long residence times and thus not be readily available to plants.

We hypothesised that increased demand for easily available P under elevated CO<sub>2</sub> due to larger poplar tree biomass would, in turn, decrease the stock of less available P. In contrast to our hypothesis, the plant available, the weatherable mineral P pool and degradable organic P pools increased under FACE. This suggests that the availability of P can actually increase in elevated CO<sub>2</sub>, forming a positive feedback with increased biomass production on P limited soils. However, the observed increase might not be a common response since *Johnson* (2004) found a decrease in easily available soil P and no change in HCl extractable P fraction in sweetgum under elevated CO<sub>2</sub>.

Using element ratios we have attempted to determine the source of the P taken up by the poplars. There were no changes in the ratio of Ca:Sr in leaves or roots under FACE compared to ambient. In our soils, the Ca:Sr ratios in the sequential extractions were also not sufficiently distinct among treatments to be useful in determining the source of any weathered elements. The largest increase in P under FACE was seen in the HCl extractable pool. This increase was accompanied by significant changes in the amount of P, Ba and Ca and in the elemental ratio of Ba:Sr in the HCl extractable fraction. The increase in total Ba and Ca seen under FACE suggests that the P in the HCl fraction may be associated with release from a mineral containing P, Ba and Ca, or that a change in soil chemistry traps these elements in this extraction fraction. *Bullen & Bailey* (2005) used Ba:Sr ratios to separate Ca uptake from organic and mineral soil horizons. Organic horizons had higher Ba:Sr ratios than mineral horizons. In the HCl fraction, under FACE the Ba:Sr ratio was higher than under ambient, which may be evidence of an organic source of this fraction. The EuroFACE site has experienced higher turnover of organic matter under FACE due to priming of old soil organic matter (*Hoosbeek et al.*, 2004). However, the turnover of P associated with the organic matter is not large enough to account for the increase in the HCl fraction (*Khan et al.*, 2008). While the element ratios do not allow us to determine the specific mineral source of P in the HCl fraction, they give a clear indication that it originates from different sources under ambient and FACE conditions (Table 3). Alongside an increase of P we have observed significant increases of Ba and Ca, as well as an increase in Ba:Sr ratio.

Both plants (*Kelly et al.*, 1998) and associated mycorrhizal fungi (*Jongmans et al.*, 1997) have been suggested to weather minerals in soils. *Blum et al.*, (2002) have shown that in Ca depleted conditions, ectomycorrhizal trees are able to access less available CaPO<sub>4</sub> pools. *Cumming* (1990) showed that ectomycorrhizal pine seedlings were able to utilize AlPO<sub>4</sub> as a P source. Both CaPO<sub>4</sub> and AlPO<sub>4</sub> are soluble in NaOH or HCl. As the P available from either biomass turnover (leaves and roots) or turnover of soil organic matter in EuroFACE was not sufficient to account for the increase in the P pool in the HCl fraction (*Khan et al.*, 2008), this further strengthens the idea that the P increase must be from weathering of more occluded pools. Much of the new soil organic matter formed at the EuroFACE site was shown to be from fungal hyphae (*Godbold et al.*, 2006). Ectomycorrhizal hyphae have been shown to contain large amounts of P (1000 mg P kg<sup>-1</sup>) primarily in the form of polyphosphates (*Hagerberg et al.*, 2005). This suggests that the HCl-extractable fractions could be linked to a biological (mycorrhizal) component in the soil. The action of roots and mycorrhizas in mineral weathering is well known, as is the modification of organic P forms due to ectoenzymes (*Sinsabaugh et al.*, 1993). Based on presented evidence, we suggest that under FACE the changes in P distribution between the extraction fractions may be a result of root and mycorrhizal mineral weathering and

organic matter mineralisation, as well as biomass turnover. These factors have resulted in either the formation of a biogenic P containing component in the soil or one present within a soil biomass component such as mycorrhizal hyphae. The turnover of plant biomass is known to result in the formation of biogenic opaline silica, which is more soluble than primary silicates (Kelly *et al.*, 1998). An alternative explanation involves an increase in soil CO<sub>2</sub> efflux. This is a common response of ecosystems to elevated atmospheric CO<sub>2</sub>, on average between 16 and 39 %. At the EuroFACE site soil CO<sub>2</sub> efflux increased by 31% to 50% (King *et al.*, 2004; Norby *et al.*, 2002). The increase in both autotrophic and heterotrophic soil respiration results in an increase of partial pressure of CO<sub>2</sub> in soil air (Oh & Richter, 2004), and subsequently in an increase in soil water carbonates (Andrews & Schlesinger, 2001). They showed that the increase in carbonic acid concentration in soil water leads to a 25% increase of concentration of Si. The soil pH at the EuroFACE site should prevent formation of carbonates, but the increase in partial pressure of CO<sub>2</sub> may affect soil chemistry. However, due to the methodological limitations of our study we could not point out the precise pathway leading to increased P availability in FACE and all of the potential mechanisms need further investigation.

This investigation shows that increased tree growth under elevated CO<sub>2</sub> has not resulted in depletion of P pools in soils, but rather in replenishment and increased storage of P in the rooting zone. Phosphorus limitation may therefore not reduce tree growth in a high CO<sub>2</sub> world. Furthermore, this work suggests that biogenically driven weathering of primary minerals in the rooting zone is sufficient to maintain the replenishment of plant available inorganic P, but leads to an increase in weatherable P pools.

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