Tansley review

Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal

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Summary

As C_3 photosynthesis is not yet CO_2-saturated, forests offer the possibility of enhanced growth and carbon (C) sequestration with rising atmospheric CO_2. However, at an ecosystem scale, increased photosynthetic rates are not always translated into faster tree growth, and in free air carbon enrichment (FACE) experiments with trees, the stimulation in above-ground growth often declines with time. So is tree growth C-limited? The evidence is reviewed here at three different scales. First, at the biochemical scale, the role of Rubisco is discussed by considering its evolution and role as a nitrogen (N) storage protein. Second, at the ecophysiological scale, C allocation to gain nutrients from the soil is considered and it is argued that any C limitation is only through a limitation to soil nutrient cycling. Finally, the response of forest ecosystems to rising atmospheric CO_2 concentrations is considered and evidence from FACE experiments is discussed. From the three lines of evidence we conclude that the growth of trees is not C-limited, with the key to understanding future responses to climate change being turnover of soil organic matter and nutrient cycling.

I. Introduction

As a consequence of burning fossil fuels and land use change, atmospheric concentrations of CO_2 are rising at a rate unprecedented in the recent history of the planet. The current CO_2 concentration of 382 µmol mol^{-1} (2006) is 38% above the preindustrial figure of 270 µmol mol^{-1} (which had been stable for thousands of years) and nearly twice as high as it was towards the end of the last ice age (Neftel *et al.*, 1988). In the face of rapidly rising atmospheric CO_2 concentrations, there is considerable interest in whether forests will grow faster and so sequester more C, thereby helping to mitigate C emissions and their consequences for global warming. Forests cover over 45% of the land surface of the Earth and account for some 75% of terrestrial net primary production (Melillo *et al.*, 1993). Therefore, as C_3 photosynthesis is not yet CO_2-saturated, forests potentially offer the possibility of enhanced growth and C sequestration as atmospheric CO_2 concentrations continue to rise. At a physiological level, elevation of CO_2 results in a direct increase in net photosynthesis, because of both an increased velocity of carboxylation and an inhibition of photorespiration (Long *et al.*, 2004). However, at an ecosystem scale, increased photosynthetic rates are not always translated into faster tree growth (Oren *et al.*, 2001) or C sequestration (Luo *et al.*, 2004) and initial stimulation in above-ground growth often declines with time (Körner, 2006). So is tree growth C-limited?

The origin of the concept of limitation is Liebig's law of the minimum (von Liebig, 1840) and was first applied to investigate the limitation on the productivity of individual plants or crops imposed by an inadequate supply of individual nutrients from managed soils. Since then, element limitation has been used to interpret differences in productivity of natural ecosystems (Chapin, 1980; Vitousek & Howarth, 1991) and, even though still focusing on the measure of plant productivity, the original concept has been developed further in attempts to overcome some of its inherent constraints in explaining the behaviour of integrated systems. The realization that not one but several resources can constrain productivity simultaneously (Chapin & Shaver, 1985) led to the concept of multielement limitation (Bloom *et al.*, 1985) and the resource optimization paradigm (Rastetter & Shaver, 1992). The concept of stoichiometry has provided a wider angle on factors controlling the flow of elements in biological systems, including plant production, in that it stresses the importance of element ratios, particularly C, N and phosphorus (P), at key interaction points of the element cycles (Hessen *et al.*, 2004). The resulting requirement to focus on the interaction between element cycles (Chapin *et al.*, 1987) and their individual time lags in responding to a change of plant resource investments, particularly when addressing links between atmospheric CO_2 and soil resources (such as N), has triggered concepts like progressive nitrogen limitation (Luo *et al.*, 2004). Hence, C limitation needs to be assessed in the context of the use of other resources by the tree.

Resource use by plants in relation to their environment is often considered using C as the basic currency. There are several reasons for this common approach. First, C is the major component of the dry matter of plants. Second, physiologists have implicitly assumed that because photosynthesis is limited by CO_2, plant functioning can therefore be considered in terms of the C 'cost'. Third, because of concerns over global environmental change, the C cycle has become a major research theme in its own right, particularly understanding the processes governing C sequestration in ecosystems. As a consequence, our knowledge of the C physiology of trees is far more advanced than that of other nutrients, and so the response of trees to environmental change is often interpreted solely in terms of C. The apparent assumption underlying this approach is that the ability of trees to assimilate and allocate C ultimately regulates their capture and use of other resources and hence growth.

Three other issues in this carbon-centric approach need to be considered. First, many of the physiological experiments have used 'optimal' conditions, rather than using conditions that impose constraints of light, nutrients or temperature. Yet these conditions of constraint are more biologically meaningful (Warren & Adams, 2004). Second, the difference between a deficiency and a limitation to tree growth needs to be stressed. Under experimental conditions where nutrient supply is carefully controlled, a nutrient deficiency (which has visible symptoms such as foliage discoloration) is easy to induce. However, outside of intensive forestry plantations such symptoms are very rare for trees growing in soils. Instead, there are fast-growing trees on fertile sites or slower-growing plants where nutrient availability is lower. However, the absence of deficiency symptoms does not in any way preclude a limitation to growth. Finally, most ecological experiments that have manipulated resource availability to trees (e.g. by elevated CO_2) have been of a relatively short duration. As a consequence, it has not been possible to consider the effect of potentially complex feedback processes that operate at the ecosystem scale and which can occur over a timescale of years to decades (Fig. 1).

To overcome some of these potential problems in interpreting experimental data, an alternative approach is to consider evidence over a range of temporal or spatial scales. Körner (2003) published a study which purportedly showed that tree growth in four climatic zones (boreal and mountain, temperate, Mediterranean and tropical) was not limited by the availability of C. He showed that, with only one exception (dry mid-summer in the Mediterranean), nonstructural carbohydrate pools (as a measure of C shortage or surplus for growth) were maximal when tree growth was reduced or zero, irrespective of the time of year. He interpreted these results as meaning that sink activity and its direct control by the environment, or developmental constraints, restricted growth, not C availability (Körner, 2003). The aim of this paper is to extend this approach by reviewing the evidence for a C limitation to tree growth and
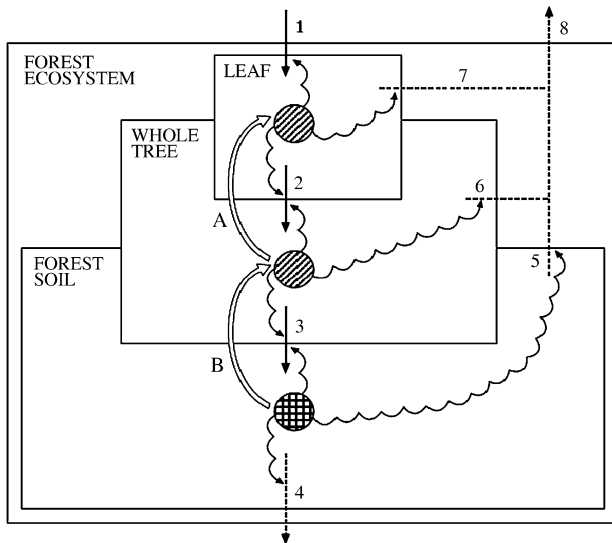
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Fig. 1 A schematic representation of the carbon (C) flux through forest ecosystems, highlighting the different scales at which the fluxes in and out are controlled. Three different scales are considered: the leaf, the whole tree (including roots, mycorrhizal fungi and their extrametrical mycelial network in the soil and bacteria in the rhizosphere of roots) and the forest soil. C inputs into the system (→) are represented by the following: 1, C assimilation by Rubisco; 2, C export from leaves to woody tissues; 3, C transfers from the whole tree to soil as litter returns, turnover of roots and mycorrhizal hyphae and exudation. C losses from the system (- - ►) are represented by the following: 4, leaching of dissolved organic C from the soil; 5, heterotrophic soil respiration resulting from the turnover of soil organic matter; 6, autotrophic soil respiration and tree respiration (excluding photorespiration); 7, leaf photorespiration. Together, 5–7 determine ecosystem respiration. 8, Within each scale, C inputs and outputs are regulated by the internal status of the compartment involved, that is, leaf and whole-tree physiological status (▨), and amount of soil organic matter and biogeochemical cycling (⊕), with feedback controls of the C status of the compartment acting upon the inputs and outputs (᷍➔). Two main feedback controls operate between scales (⇄): between the leaf and whole-tree scale through physiological processes operating over a timescale of seconds to days (A); and between the soil and whole tree, through processes operating at a timescale of hours to decades (B).

challenge the 'carbon-centric' approach by considering the response of trees to environmental change. Evidence for C limitation will be considered at three different scales (Fig. 1). First, photosynthetic responses at the leaf scale will be considered in relation to the evolution of the carboxylation activity of Rubisco and the role of the protein in N storage. How Rubisco is affected by elevated CO_2 will also be considered. Second, at the whole-tree scale, C storage and allocation to roots and associated microbes in order to gain nutrients will be considered and it is argued that any C limitation is only through a limitation to soil nutrient cycling. Finally, we will consider how forest ecosystems are responding to rising atmospheric CO_2 concentrations and the soil feedback processes involved.

II. The biochemical scale: the paradox of Rubisco

Rubisco is the most abundant enzyme on the Earth and has two different catalytic functions: catalysing the initial step of the carbon reduction and oxidation cycles, using CO_2 and O_2 as the substrates, respectively. The two cycles work against each other as the oxidation cycle (photorespiration) uses light energy to release recently assimilated C as CO_2. Overall photorespiration decreases the net efficiency of photosynthesis by 20–50%, depending upon temperature (Zelitch, 1973). Compared with the other enzymes in the Calvin cycle, Rubisco is inefficient at carboxylation, with a much lower substrate affinity for CO_2 than the other enzymes for their substrates. As C_3 photosynthesis is not saturated at current (rising) atmospheric concentrations of CO_2, if trees were C-limited, why has there not been a strong evolutionary pressure to increase the efficiency of the carboxylation function of Rubisco during the last 26 million yr, during which atmospheric CO_2 concentrations have been lower than at present (Pearson & Palmer, 2000)? This paradox is discussed in relation to C limitation in trees by considering the role of Rubisco as a storage protein, the evolution of CO_2 concentrating mechanisms in plants and the response of Rubisco in elevated CO_2 studies.

1. The role of Rubisco as an N storage protein

The model of Farquhar *et al.* (1980) predicts that C_3 photosynthesis is limited by the amount of Rubisco and is borne out by the majority of physiological studies which have genetically manipulated the amount of Rubisco in (mainly crop) plants (reviewed by Parry *et al.*, 2003). However, we now know that despite its relative inefficiency at C assimilation, trees can have Rubisco concentrations in excess of that needed for photosynthesis (Warren *et al.*, 2000, 2003; Warren & Adams, 2001; Table 1). The catalytic activity of Rubisco is under complex regulation involving an activase enzyme (Andersson & Taylor, 2003) and at any point in time there are two pools of Rubisco in a leaf, comprising activated and inactive protein. The balance between the two can be modified by N supply, with an increased leaf N status increasing the amount of Rubisco protein, but causing a decline in the Rubisco activation state (Cheng & Fuchigami, 2000; Manter *et al.*, 2005).

Accumulation of an excess of Rubisco in response to a generous N supply is because Rubisco has a third function, as a N storage protein. This has been demonstrated in herbaceous plants (Millard, 1988; Quick *et al.*, 1992) and is common in species that have monocarpic senescence, N being released from Rubisco in older (shaded) leaves as they senesce, providing N for both new leaf growth and reproduction (Millard & Catt, 1988; Suzuki *et al.*, 2001). There is evidence that in a range of both deciduous and coniferous, evergreen trees, Rubisco can temporarily store a significant proportion of the N that is used subsequently for leaf growth (Table 2). For deciduous species in temperate ecosystems, for example, this

Table 1 The effect of autumn nitrogen (N) supply to Scots pine on the number of current season needles, their dry weight, nitrogen and Rubisco content, and their photosynthetic capacity

	N supply after flushing		
Needle parameter	No N	Low N	High N
Needle number (per tree)	1698 ± 423 a	1566 ± 324 a	1493 ± 132 a
Needle dry weight (mg per needle)	10 ± 1.9 a	10 ± 2.0 a	14 ± 2.9 a
Needle N content (μg per needle)	95 ± 20 a	185 ± 32 b	350 ± 38 c
Rubisco content (mg m^{-2})	82 ± 29.8 a	770 ± 180 b	1506 ± 184 c
Photosynthetic capacity (μmol m^{-2} s^{-1})	8.3 ± 1.23 a	12.7 ± 3.43 a	9.3 ± 2.87 a

Each value is the mean and standard error of five replicate trees; different means across rows ($P < 0.05$) are postmarked with different letters. Values within a row having the same letter are not statistically significantly different ($P < 0.05$).
Three-year-old seedlings of *Pinus sylvestris* were grown in sand culture in individual pots with a N supply of 200 cm^3 of a nutrient solution containing 2 mol NH_4NO_3 m^{-3} three times a week from 14 May until 31 July, by which time terminal resting buds had been set. From 1 August to 22 November, the trees received 200 cm^3 of nutrient solution three times a week but containing 0 (no N), 2 (low N) or 8 (high N) mol NH_4NO_3 m^{-3}. The photosynthetic capacity of the current year's needles was measured at saturating light conditions (1500 μmol m^{-2} s^{-1}) and at a leaf temperature of 18°C, and the needles were then harvested and counted and the N, Rubisco content (using the method of Catt & Millard, 1988) and dry weight measured.

Table 2 Summary of the evidence for Rubisco acting as a nitrogen (N) storage protein in trees, along with those studies that found no evidence for such a role for the protein

Evidence	Species	Leaf habit	References
Studies finding evidence for Rubisco as an N store			
Over-investment in Rubisco for photosynthetic requirements	Sclerophyllous shrubs	Evergreen	Warren *et al.* (2000)
	Pinus pinaster	Coniferous evergreen	Warren & Adams (2001)
	Pinus sylvestris	Coniferous evergreen	Warren *et al.* (2003)
	Pinus sylvestris	Coniferous evergreen	P. Millard (unpublished)
Preferential loss of Rubisco N from leaves during senescence	*Malus domestica*	Deciduous	Kang & Titus (1980)
Loss of Rubisco N during leaf senescence providing 30–48% of N used for leaf growth the following year	*Malus domestica*	Deciduous	Millard & Thomson (1989)
Specific decrease in Rubisco protein from 1-yr-old needles during flushing	*Pseudotsuga menziesii*	Coniferous evergreen	Camm (1993)
Rubisco activation state decreases with increasing leaf N status	*Malus domestica*	Deciduous	Cheng & Fuchigami (2000)
	Pseudotsuga menziesii	Coniferous evergreen	Manter *et al.* (2005)
Studies finding no evidence for Rubisco as an N store			
Loss of N, but not Rubisco, from old leaves to support new leaf growth	*Eucalyptus globulus*	Broadleaf evergreen	Wendler *et al.* (1995)
	Nothofagus fusca	Broadleaf evergreen	Stephens *et al.* (2001)
	Eucalyptus globulus	Broadleaf evergreen	Warren (2004)

allows a sink for sustained N uptake in summer, once leaf growth has finished, continuing into autumn while leaves senesce (Millard & Thomson, 1989). Coniferous, evergreen trees store N in their youngest class of needles once a terminal bud has been set (Millard & Proe, 1993; Millard *et al.*, 2001), allowing for continued N accumulation (as Rubisco) in the autumn, without growth or enhancement of needle photosynthetic capacity. This was demonstrated in an experiment where young Scots pine trees were given contrasting N supplies once shoot terminal buds had been set and shoot extension growth finished for the year (Table 1). Needle number per tree, needle size and photosynthetic capacity were unaffected, whereas needle N content increased and Rubisco concentrations rose nearly 20-fold in response to a generous autumn N

supply (Table 1). In coniferous, evergreen trees, N remobilization from Rubisco occurs during flushes of growth, without needle senescence (Table 2). Taking a 'carbon-centric' view, it makes no sense that a plant would store N in Rubisco, given the large energy costs associated with the synthesis of such a complex protein and the need to maintain the machinery for its regulation. However, using Rubisco as an N store compared with either nitrate or amino acids has the advantage of allowing large amounts of N to be stored without osmotic or toxic consequences (Millard, 1988). Also, unlike most other proteins, Rubisco has a very slow turnover rate (and therefore maintenance costs) unless the plant is stressed and leaf senescence is triggered (Suzuki *et al.*, 2001). It is also important to stress that the ability to store N in Rubisco is not evidence of

N sufficiency, as physiologically this will be more important for N-limited than N-replete plants (Millard, 1988) and so will occur without there necessarily being a surplus of Rubisco for photosynthesis.

2. Evolution of Rubisco and CO_2 concentrating mechanisms

Understanding the role of Rubisco as a storage protein is important, when considering if a potential C limitation to trees has resulted in an evolutionary pressure to increased its carboxylation efficiency. Isolation of Rubisco from a diverse range of species and comparison of the relative specificity of the enzyme for its two substrates, CO_2 and O_2 (S_{rel}), has shown differences, suggesting that natural selection for increasing the carboxylation function has occurred. However, a higher specificity for CO_2 seems to be acquired at a cost, as the catalytic efficiency of Rubisco (K_{cat}; mol CO_2 mol Rubisco active sites s^{-1}) decreases (Bainbridge *et al.*, 1995). K_{cat} has also been shown to vary amongst C_3 species in relation to temperature, with species from a cool climate having a higher K_{cat} (Sage, 2002), thereby potentially increasing their photosynthesis and photosynthetic N use efficiency. Sage (2004) suggested that the relatively narrow range of S_{rel} found in C_3 plants reflects a balance for selection for enhanced S_{rel} and high K_{cat}. So, in the absence of further improvements to Rubisco, several examples of CO_2 concentrating mechanisms have evolved in higher plants, such as C_4 photosynthesis and crassulacean acid metabolism (CAM). These adaptations have allowed for an increased carboxylation efficiency of Rubisco and thus a smaller investment in the protein.

C_4 photosynthesis involves fixation of CO_2 by PEP carboxylase in mesophyll cells. This is followed by decarboxylation of the C_4 acids in specialized bundle sheath cells, thereby elevating CO_2 concentrations up to 10-fold, so that it is saturated for reassimilation by Rubisco (von Caemmerer & Furbank, 2003), thereby suppressing photorespiration. This adaptation appears to have evolved over 45 times independently within 19 families of angiosperms (Sage, 2004). It has been suggested that one of the main drivers for the evolution of C_4 photosynthesis was decreasing atmospheric $[CO_2]$ (Ehleringer *et al.*, 1997), coupled with rising O_2 concentrations (Sage, 2004). CAM also involves the fixation of CO_2 by PEP carboxylase (with the resultant organic acids often being stored in vacuoles) followed by decarboxylation of organic acids for assimilation by Rubisco (Lüttge, 2004). There is a great plasticity in the CAM adaptation to photosynthesis, with some species fixing atmospheric CO_2 at night whilst the stomata remain closed during the day, and others fixing atmospheric CO_2 throughout the 24 h period (Dodd *et al.*, 2002).

However, in relation to trees, neither of these adaptations is widespread. C_4 photosynthesis is found in some woody shrubs and small trees but no large trees, while CAM is found only in neotropical trees of the genus *Clusia* (Lüttge, 2006).

Both C_4 and CAM pathways are relatively recent adaptations of the ancestral C_3 pathway. In both these cases, the amount of Rubisco needed by the plant has been decreased and these adaptations are found predominantly in ecosystems where N availability is not the major limitation to photosynthesis or growth. C_4 plants are found predominantly in warm temperate, subtropical and tropical regions at higher temperatures (Ehleringer *et al.*, 1997), where P availability is often a greater limitation than N. CAM plants are found predominantly as succulents in arid environments or as epiphytes in rainforests and probably evolved as a consequence of multiple environmental stresses, including salinity, nutrients and water (Lüttge, 2004). As a consequence, the fact that both CAM and C_4 mechanisms result potentially in a smaller storage capacity for N is unlikely to affect plant growth or competition.

The answer to the paradox of the continuing Rubisco catalytic inefficiency is probably its role as a N storage protein. The first appearance of Rubisco in terrestrial plants was some 500 million yr ago, when atmospheric CO_2 concentrations were much higher than at present and O_2 concentrations lower. This probably explains its low affinity for CO_2. Perhaps as atmospheric CO_2 concentrations fell, plants were still not C-limited as, overall, there was a greater nutrient limitation. As already noted, young soils (in geological time scales) probably had very slow mineral weathering rates and had little input of N from N_2 fixation, causing an overall N limitation (Chadwick *et al.*, 1999). In young, highly leached soils, P limitation can also quickly develop (Richardson *et al.*, 2004). As a consequence of nutrient limitations, the inefficient enzyme for carboxylation did not need to evolve further, as it was also useful as an N store. The fact that trees (unlike many other higher plants) have not utilized C_4 or CAM-type adaptations to photosynthesis suggests that there was little pressure to assimilate more C. Even if photosynthesis is limited by the amount of Rubisco, it does not necessarily follow that in long-lived plants such as trees, which assimilate C over decades, growth is C-limited. Thus at the biochemical scale, considering the roles of Rubisco, it would appear that there is little evidence that the current atmospheric CO_2 concentration is a major limitation to the functioning of trees. So, given the implication from the functioning of Rubisco, that plants in general are more nutrient- than C-limited, are the biochemical responses of tree leaves (and Rubisco in particular) to elevated $[CO_2]$ consistent with the hypothesis that trees are more nutrient- than C-limited?

3. Rubisco and elevated CO_2

In most species studied, elevated CO_2 has two primary effects. First, increased net C assimilation rates are the result of both increased carboxylation and competitive inhibition of oygenase reaction, thereby decreasing C losses through photorespiration. Second, there is often an effect upon stomatal aperture to reduce stomatal conductance, thereby increasing the water

use efficiency of photosynthesis. Some studies have reported an initial stimulation in net photosynthesis slowing down or disappearing after a few years (Roberntz & Stockfors, 1998; Griffin *et al.*, 2000). The reason for this is photosynthetic acclimation, which can be defined as 'any adjustment in the C acquisition system that may develop over time in plants grown continuously in elevated CO_2' (Wolfe *et al.*, 1998). This often involves down-regulation of the Rubisco protein, accompanied by a change in the balance of its active vs inactive forms, and is a particularly common response under low-N conditions (Stitt & Krapp, 1999).

In a meta-analysis of data from a range of European experiments growing trees with elevated CO_2, Medlyn *et al.* (1999) examined photosynthetic model parameters. Using the Farquhar model of photosynthesis, they found that under elevated CO_2 there was a down-regulation of potential electron transport rate (J_{max}) and maximum Rubisco activity ($V_{c\,max}$) of the order of 10%, but found little evidence (only one study out of six) for a downwards shift in the relationships between J_{max}, $V_{c\,max}$ and leaf N concentration. They concluded that down-regulation of photosynthesis had several causes: reduced leaf N (because of a limited N availability compared with increased C availability) and a shift in the relationship between photosynthetic parameters and leaf N, probably as a result of an accumulation of nonstructural C in the leaves (Medlyn *et al.*, 1999). Using a similar approach, Rogers & Humphries (2000) calculated the minimum $V_{c\,max}$ capable of supporting acclimated photosynthetic rates observed under elevated CO_2. They found a strong correlation between observed and predicted values, which they interpreted as $V_{c\,max}$ and investment in Rubisco being coupled to requirements for C assimilation (Rogers & Humphries, 2000), again suggesting that as there is a loss of active Rubisco under elevated [CO_2], the trees were not C-limited. The view based on findings from the free-air carbon dioxide enrichment (FACE) experiments is that there is a selective loss of Rubisco enzyme under elevated CO_2, benefiting N use efficiency (Long *et al.*, 2004), without there necessarily being much change in leaf C assimilation rate.

Thus in N-limited ecosystems, the key effect of the plants' response to elevated CO_2 is not removal of a limitation but an increase in efficiency of N use for photosynthesis (Drake *et al.*, 1997). This view might well not be correct in the medium term if less investment in Rubisco protein compromises the ability to store and internally cycle N for growth. However, while this has never been studied experimentally, it is interesting that the acclimation of Rubisco to increased [CO_2] in 1-yr-old needles of pines was not found in the current year's needles (Turnbull *et al.*, 1998; Tissue *et al.*, 2001; Rogers & Ellsworth, 2002). As current needles are the main site of N storage in pines (Millard *et al.*, 2001), this suggests that acclimation does not occur until after Rubisco turnover to release N from storage from the youngest needles at the onset of spring growth. Acclimation would therefore not occur at the expense of N storage by the tree.

III. The ecophysiological scale: the profligate use of carbon

Trees are profligate in how they use and lose C. There are complex feedbacks between C inputs from trees to soil and nutrient cycling processes regulating soil fertility (Fig. 2). As a consequence, trees allocate a lot of their C in order to gain nutrients, but have evolved elaborate mechanisms for conserving, storing and internally cycling nutrients. Evidence for a C limitation for tree growth will be discussed, by considering C allocation at the whole-tree scale in relation to the persistence of soluble carbohydrate pools in perennial tissues, suggesting that much of the C is sequestered and not stored; leaf senescence and the efficient withdrawal of nutrients compared with carbohydrates; and allocation of C below-ground to roots and associated microbes to acquire nutrients.

1. Carbon storage or sequestration?

Storage (as defined by Millard, 1988) involves C being held in a pool, with the potential to be reused subsequently for growth or maintenance of another. In contrast, sequestration represents a metabolic dead end, with the C no longer physiologically active and so not affecting metabolism. So do trees store C? The short answer is yes, but much of the nonstructural soluble carbohydrates (NSC), such as starch, that are accumulated in woody tissues are sequestered and so probably not reusable by the tree. Seasonal fluctuations in the concentrations of NSC such as starch have also been found in many species, especially in seedlings (Gansert & Sprick, 1998). The ability to store C (and nutrients such as N and P) is important in allowing trees to recover from disturbances such as defoliation, and the relative ability to store C has been associated with seedling survival and growth in temperate (Kobe, 1997), tropical (Gleason & Ares, 2004) and boreal species (Kagawa *et al.*, 2006a). C remobilization in deciduous trees has also been demonstrated as an important process during bud burst (Maurel *et al.*, 2004), providing up to about 40% of the C used for new leaf growth in a boreal species with a short growing season (Kagawa *et al.*, 2006a) and contributing to early wood formation (Kagawa *et al.*, 2006b). C remobilization can also be important for recovery from winter embolism (Ameglio *et al.*, 2004).

Trees certainly accumulate large pools of NSC. Hoch *et al.* (2003a) estimated that in a temperate forest the concentrations of NSC found in mature trees would allow the whole leaf canopy to be replaced four times. Even when tree growth is constrained to a short season by temperature (e.g. at the tree line) the build-up of NSC suggests that C availability is not a limitation to growth (Hoch & Körner, 2003). Furthermore, NSC pools in trees are never fully depleted in the same way that N storage pools are (e.g. Hoch *et al.*, 2003a). Even girdling the phloem does not deplete NSC in roots completely (Jordan & Habib, 1996; Bhupinderpal-Singh *et al.*, 2003), suggesting
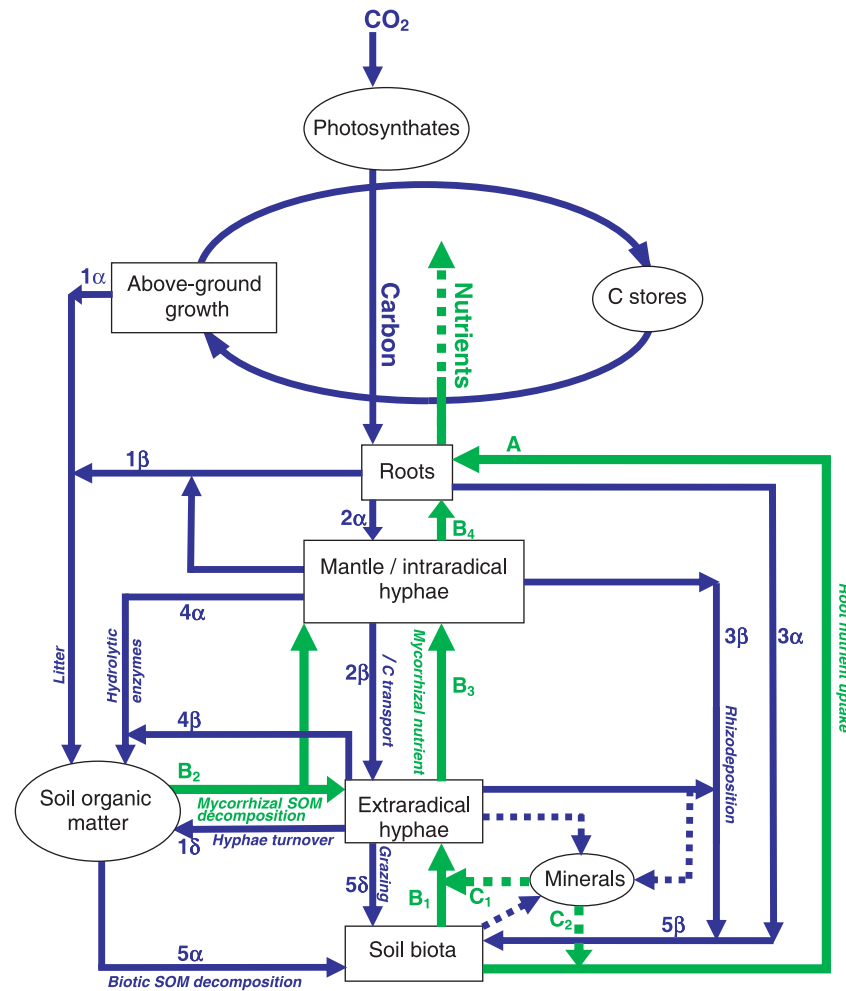
Fig. 2 A schematic representation of carbon (C) inputs from a tree to soil and the consequent pathways for nutrient acquisition by the roots. The pathways of C input into soil are as follows: (1) via biomass turnover through leaf senescence (1α), root senescence (1β) and turnover of extraradical mycorrhizal networks (1δ); (2) C transfer from roots to mycorrhizal hyphae; (3) rhizodepostion by nonmycorrhizal (3α) and mycorrhizal (3β) roots, influencing soil biota and mycorrhizal saprophytic activities; (4) secretion of enzymes by the mantle/intraradical hyphae (4α) and extraradical hyphae (4β) which break down soil organic matter (SOM); (5) C uptake by soil biota (i.e. all soil organisms except mycorrhizal fungi) through decomposition of soil organic matter (5α), consumption of rhizodeposits (5β) and grazing of mycorrhizal hyphae and roots (5δ). The pathways for nutrient return to the roots which are fuelled by the C inputs are as follows: A, root uptake of mineral and low-molecular-weight soluble organic N/P compounds produced by soil biota; B_{1-4}, transfer of N/P from mycorrhizal partners to tree roots, through competition with roots to take up soluble nutrients released by soil biota (B1), or utilization of complex sources of organic N/P (B2), which can then be utilized for fungal growth or transported and used across the mycorrhizal network (B_3) and/or transferred to the roots (B_4). In addition, there is mineral weathering (C) and subsequent uptake of nutrients by mycorrhizal fungi (C_1) or directly by roots (C_2). The potential influence of rhizodeposits, soil biota and/or mycorrhizal hyphae is indicated by dotted lines and could be mediated via C compounds and/or other physical and chemical factors.

that roots contain pools of NSC which are not utilized, even when the supply of current assimilates is cut. So is the build-up of NSC in leaves, trunks and roots of trees C storage or sequestration? It seems to be partly a mechanism to avoid down-regulation of photosynthesis, sequestering C that cannot be reused by the tree. This is a mechanism comparable to arginine accumulation in conifers in response to excessive N deposition (Näsholm, 1994).

In comparison, it is well established that trees store nutrients (Millard, 1996) and that, particularly for N, remobilization of stored resources can provide the first source used for growth during flushing and the majority used for growth above ground each year (Dyckmans & Flessa, 2001; Millard *et al.*, 2001; Carswell *et al.*, 2003). N remobilization from storage is source-driven (Millard *et al.*, 2001). In consequence, in contrast to C in NSC pools, all the stored N is depleted during

remobilization, as has been seen in temperate species by, for example, the complete disappearance of bark storage proteins during the spring (Wetzel *et al.*, 1989; Cooke & Weih, 2005).

2. Leaf senescence

Nutrient withdrawal from leaves during senescence is often very efficient and in deciduous trees plays an important role in their internal cycling of N and P (Millard, 1996). The remaining nutrient fraction returns to the soil as litter, with the mass and nutrient content of litter produced being the primary determinant of nutrient availability in forest soils (Prescott, 2002). The fraction of mineral nutrients withdrawn from leaves before their abscission is variable with differences associated with leaf life span, overall leaf nutrient content, soil fertility (Niinemets & Tamm, 2005) and environmental conditions such as drought (Del Arco *et al.*, 1991). In a review comparing over 100 deciduous shrubs and trees, Aerts (1996) reported that $54 \pm 16\%$ of the N and $50 \pm 20\%$ of the P was withdrawn from a leaf, with resorption efficiencies usually lower for trees growing on sites with higher fertility. Van Heerwaarden *et al.* (2003) suggested that these values could be increased by a further 6–10% when accounting for changes in leaf mass and areas during senescence, the implication being that the majority of leaf nutrients were reused by the tree. In addition, trees have also evolved pigment systems (such as anthocyanins) to protect leaves from radiation damage and so facilitate nutrient recovery during senescence (Hoch *et al.*, 2003b). One of the first events to occur during senescence is a turnover of Rubisco and export of N (as amino acids) from the leaf. This selective breakdown of Rubisco can lead to an approximately 80-fold greater rate of turnover than of other enzymes of the Calvin cycle (Crafts-Brander *et al.*, 1990), thus emphasizing the role of Rubisco as a N storage protein.

In contrast to nutrient withdrawal from senescing leaves, much of the soluble C present at the end of the summer remains in the abscised leaf. Indeed, leaf senescence is now thought to be triggered by the accumulation of both soluble sugars and starch in the leaf (Ono *et al.*, 2001; Pourtau *et al.*, 2004) and it is the water-soluble C content (i.e. sugars) that may determine initial litter decomposition rate (Allison & Vitousek, 2004). In the longer term at the community scale, leaf content in secondary C compounds (such as lignin, polyphenols and tannins) is negatively correlated with litter decomposition rate. As slow-growing trees tend to produce leaves rich in such compounds, the feedback between leaf quality and decomposition rate contributes to maintaining lower levels of soil fertility and is thought to explain the dominance of such trees in their habitat (Aerts, 1999; Cornelissen *et al.*, 1999). Hence C in leaf litter, in the form of complex secondary compounds or sugars, plays an important part in determining the rate of soil nutrient cycling. C investment in secondary C compounds is usually regarded as a means to protect the key organs in C acquisition against herbivory.

However, many evergreen tree species store N in their leaves and, in their case, antiherbivory compounds may serve primarily to protect the nutrients stored within the leaves (Millard *et al.*, 2001). Thus trees tend to protect and then reuse the nutrients held in their leaves, while allowing much of the soluble leaf C to abscise with the leaf. Taken together, these physiological and structural plant mechanisms suggest an evolutionary pressure led by nutrient rather than C limitation.

3. The importance of C allocation to roots and associated microbes

In addition to the C and nutrients returned to the soil as litter, trees allocate much of their C below ground. Giardina *et al.* (2005) strikingly summarized the importance of below-ground C allocation: half of the 120 Pg C fixed annually by terrestrial plants is allocated below ground, with tree-based ecosystems accounting for most of this flux, amounting to 20 times the annual release of C by combustion of fossil fuels. Below-ground C allocation has been estimated as being between 35 and 50% of net primary production (NPP) in a tropical forest (Giardina *et al.*, 2003), and as much as 73% in Douglas fir (Fogel & Hunt, 1983) and black spruce (Ruess *et al.*, 2003) forests. Figure 2 shows the pathways for C input to the soil: from litter (pathway 1); by transfer from roots to mycorrhizal fungi (pathway 2); directly into the soil as mycorrhizodeposits (pathway 3) or secreted enzymes (pathway 4); and through grazing by soil fauna (pathway 5). The profound impact of this C (particularly through pathway 2 in Fig. 2) on the functioning of soil microbial communities has been demonstrated in large-scale field experiments. Girdling trees has been used to stop the flow of photosynthates to roots (Högberg *et al.*, 2001), thereby reducing soil respiration. In a tropical eucalyptus forest, girdling reduced soil respiration by 16–24% (Binkley *et al.*, 2006), despite tree canopies remaining intact for 3 months and live fine root biomass showing no decrease for at least a further 2 months, indicating C storage capable of sustaining root maintenance and respiration. Girdling lowered soil respiration by more than 50% in forests in northern Sweden (Bhupinderpal-Singh *et al.*, 2003) and Germany (Subke *et al.*, 2004) and 31–44% in Colorado (Scott-Denton *et al.*, 2006). Furthermore, there was an average decline of 32% of the soil microbial biomass within 3 months at the Swedish site, attributed by Högberg & Högberg (2002) to a loss of the extra radical ectomycorrhizal mycelium.

The formation of associations with mycorrhizal fungi is ubiquitous in the plant kingdom. Roots of forest trees are heavily colonized by ectotrophic (ectomycorrhizal, EM) and/ or endotrophic (arbuscular mycorrhizal, AM) fungi. For example, Ruess *et al.* (2003) found 100% of first-order roots to be EM in three mature black spruce forests, whilst Adams *et al.* (2006) reported colonization rates by EM fungi of between 76 and 100% of root length in eucalyptus forests. Gehring & Connell (2006) reported a colonization rate of up

to 61% of root length of seedlings in tropical and subtropical rain forests. A meta-analysis of data for root colonization by AM fungi found an average (across all plant species) of 36 ± 10, 23 ± 3 and $24 \pm 8\%$ root length in tropical, temperate and boreal forests, respectively (Treseder & Cross, 2006). Boreal and temperate forest trees predominantly associate with EM species, whilst the majority of forest trees growing in tropical climates have AM symbioses. There are, of course, exceptions to this crude classification, with a minority of species forming associations with EM fungi in tropical forests (Alexander, 2006) or AM fungi in temperate forests (e.g. sugar maple, sweetgum, some eucalypts and poplars). When co-occurring in the same ecosystem, EM and AM fungi have been shown to occupy different niches, with a greater abundance of EM species in organic soil horizons, and with AM species predominating in mineral horizons (Neville *et al.*, 2002; but see Moyersoen *et al.*, 1998). Some species also exhibit successional mycorrhizal associations, often with an increase in the abundance of EM and a decrease of AM fungi as the trees age, as reported by Adams *et al.* (2006) in their comparison of field-grown seedlings with mature *Eucalyptus grandis* trees. Similarly, Egerton-Warburton & Allen (2001) found a shift in the relative abundance of AM and EM fungi in oak trees along a 1–30 yr age sequence (presumably as tree carbohydrate storage capacity increased) and suggested that C supply to the roots partly controls succession from AM to EM, adding weight to the view that EM and AM fungi exhibit different C sink strengths (Lynch & Whipps, 1990). So how much C is actually transferred from roots to EM and AM mycorrhizal fungi?

Microcosm studies of EM conifers have shown that up to 30% of total photoassimilates are transferred to the fungal partner (reviewed by Söderström, 2002). Soil C balance calculations for a field site in a northern hardwood forest estimated that 17% of total below-ground C allocation was to mycorrhizal fungi (in a mixed AM and EM community) and exudation (Fahey *et al.*, 2005). However, to our knowledge, there has been no direct quantification *in situ* of the total C flux between tree roots and their mycorrhizal partners. The main impediment to direct measurements of C allocation to mycorrhizal fungi is a lack of adequate techniques. C transfer to mycorrhizal fungi can be considered as two fluxes: C exchange at the root–fungus interface (flux 2α, Fig. 2) and C allocation between intra- and extraradical hyphae (flux 2β, Fig. 2). The quantification *in situ* of C transfer at the plant–fungus interface (flux 2α, Fig. 2) is virtually impossible, without either full characterization of transport systems and specific compounds involved in resource transfer, or identification and isolation of a pool of fungal/plant specific compounds with relatively rapid turnover (such as nucleic acids) which could be used as markers of C incorporation. This second approach, together with the rapid development of molecular techniques, may allow study of both function and structure of the mycorrhizal community intercepting plant C (Johnson *et al.*, 2005a). This would be

particularly relevant, given emerging evidence that the structure of the EM community is affected by treatments changing C supply below ground (Fransson *et al.*, 2001; Parrent *et al.*, 2006). Root-excluding mesh cores, allowing ingrowth of mycorrhizal mycelia, combined with isotopic C labelling, have been used to quantify the flux of C to extraradical mycelia of AM fungi in grassland ecosystems (Johnson *et al.*, 2002), hence describing the flux of C transfer from intraradical to the network of extra radical hyphae (flux 2β, Fig. 2) which can extend centimetres (AM) to metres (EM) into the soil and link plant roots together (Selosse *et al.*, 2006). Such an approach has been used in forest ecosystems to measure fungal biomass, but not for a full C budget, because C loss through fungal respiration was not measured (Godbold *et al.*, 2006). Another major limitation to establishing an accurate C budget of the mycorrhizal community in forest ecosystems is our ability to measure mycorrhizal biomass accurately; particularly to distinguish extraradical mycorrhizal hyphae from mycelia of saprotrophic species (Wallander, 2006). Extraradical hyphae may contribute substantially to soil C stock. It has been estimated that over 60% of the accumulation of soil organic C at a poplar FACE site was derived from external mycorrhizal hyphae, twice that from leaf litter (Godbold *et al.*, 2006). In terms of litter production (flux 1δ, Fig. 2), such large amounts of fungal biomass in soils may have important implications for rates of soil organic matter (SOM) decomposition, particularly when comparing EM- and AM-dominated forests. The two types involve fungal species with fundamentally different morphology and chemical composition, which potentially affects their palatability to soil fauna. This will have inevitable consequences on the substantial amounts of C (Johnson *et al.*, 2005b) going from mycorrhizal fungi to the soil food web (flux 5δ, Fig. 2). Hyphal turnover rates also differ between AM and EM (Staddon *et al.*, 2003; Treseder *et al.*, 2004), and AM produce substances (e.g. glomalin) which can stabilize soil aggregates (Rillig & Mummey, 2006). The chemical and morphological characteristics of mycorrhizal hyphae may also affect the fine root decomposition rate. When investigating the decomposition rate of EM vs nonmycorrhizal pine roots, Langley *et al.* (2006) showed that EM roots lost only one-third of the C of nonmycorrhizal roots. Langley & Hungate (2003) hypothesized that mycorrhizal type may 'substantially influence fine root decomposition and soil carbon processing rate'.

So why do trees allocate such a substantial proportion of their C below ground and specifically to mycorrhizal fungi? The common view is that through their mycorrhizal partners, trees access nutrients otherwise unavailable for direct root uptake (such as complex forms of organic P and N), or present in insufficient quantities in the vicinity of the root (e.g. orthophosphate; Hinsinger, 2001). For example, using a microcosm system, Brandes *et al.* (1998) calculated uptakes of 73% of tree N and 76% of P via the EM fungus *Paxillus involutus* colonizing the roots of Norway spruce. In addition, van Breemen *et al.*, 2000) suggested that external hyphae play a substantial

role in nutrient return to the tree, via mineral weathering (Fig. 2, pathway C). Read (1991) hypothesized that there is a link between plant biome, mycorrhizal type and overall SOM amount, soil pH and N : P status. Read's hypothesis, later reinforced by Read & Perez-Moreno (2003), explains both the broad relative distribution of both mycorrhizal types and the body of evidence showing that AM fungi contribute predominantly to their host P requirements (but see Hodge *et al.*, 2001) through enhancement of phosphorus uptake, while EM fungi have a greater capability to mobilize N and P from SOM. In boreal forests, this could satisfy a considerable proportion of the annual nutrient requirement of the trees (Read & Perez-Moreno, 2003). Recently, understanding the functional difference between AM and EM might have been taken a step further. Lindahl *et al.* (2007) demonstrated that saprotrophic fungi were mainly found in the soil horizons where primary decomposition of recent leaf litter occurs, whilst EM fungal taxa dominated the underlying horizon where N was mobilized from partly decomposed litter. Their study added weight to the view that AM forests (tropical) have an extravagant nutrient cycle relying heavily on N mineralization, whereas EM forests (temperate/boreal) have a conservative nutrient cycle where trees access organic N via EM fungi, bypassing mineralization processes by free-living microbes (Chapman *et al.*, 2006).

It may be assumed that the 'C cost' of nutrient acquisition through symbiotic partners is higher than that of acquiring nutrients through direct root uptake from the soil solution. Does this imply that the C demand by the symbiotic partner leads to C limitation of tree growth? The consensus view is that this is not the case. Mycorrhizal infection has been shown to cause an up-regulation of photosynthesis in young EM trees in microcosms (Loewe *et al.*, 2000) and pots (Wright *et al.*, 2000) and young AM trees (Lovelock *et al.*, 1997) in pots, suggesting that when the mycorrhizal associations impose an extra C demand on the tree, it can be met by increasing C assimilation. The initial presence of the fungi may lead to a growth depression for trees under conditions of high soil fertility, as shown in field experiments in highly managed agrosystems (Graham & Eissenstat, 1998); presumably as the benefit to the plant of the increased nutrient availability conferred by the fungus diminishes. However, such levels of soil fertility are not found in forest ecosystems. To assess the C cost to the tree of nutrient acquisition via mycorrhizal fungi *in situ*, we need to understand the regulation of C exchange at the plant–root interface by both biological (e.g. plant C and nutrient status) and environmental factors (e.g. nutrient availability in the mycorrhizosphere, temperature). This is assuming that the amount of C passed on to its symbionts (2α, Fig. 2) is mainly controlled by the plant itself. However such a phytocentric view is now being questioned, and it is argued that control of C use and allocation (flux 2β, Fig. 2) by mycorrhizal partner(s) also need to be considered (Lindahl *et al.*, 2002; Staddon, 2005), especially given the extent of extraradical

mycorrhizal hyphae produced by EM fungi. In a meta-analysis comparing the response ratio of AM/EM plants (not exclusively trees) and mycorrhizal fungi to elevated CO_2, Alberton *et al.* (2005) reported evidence 'for the mycocentric view in EM, but not in AM systems'.

In addition to transferring C to their root symbionts, trees lose C from their roots as rhizodeposits (pathway 3, Fig. 2). Rhizodeposition involves a wide range of compounds (Grayston *et al.*, 1997), including exudation of low-molecular-weight substances such as sugars, organic acids and amino acids. The availability of these C substrates is considered the factor most limiting to the growth of free-living soil microbes (Wardle, 1992), explaining the greater microbial activity in rhizosphere compared with bulk soil. There are few reliable quantitative estimates of the flux of C entering the soil as exudates. A review of whole-plant ^{14}C-labelling studies performed in soil on a wide range of plant species suggested that exudation accounted for 5–10% of net C assimilation (Farrar *et al.*, 2003), although Jones *et al.* (2004) highlighted a possible overestimation resulting from methodological bias and suggested that a true estimate of root exudation was likely to be only 2–4% of net fixed C. Under a variety of stress conditions (including nutrient or water stress) the C flux from roots as exudates is increased, mainly because of damage to membranes or disruption of normal cell metabolism (Neumann & Römheld, 2001). As noted by Jones *et al.* (2004), the vast majority of studies quantifying root exudates have not considered the quantitative or qualitative impact of the (ubiquitous) mycorrhizal fungi colonizing roots (Fig. 2, pathway 3β), despite numerous studies demonstrating exudation of hydrolytic enzymes by mycorrhizal mycelium (mainly EM fungi). Recently Phillips & Fahey (2006) calculated that microbial biomass, C/N mineralization rates and phosphatase activity were 25–30% higher in the rhizosphere than in bulk soil under EM tree species. Under AM trees, rhizosphere and bulk soil differed by only 10–12% for similar parameters. Tree C inputs to soil as rhizodeposits can also act as primers for the degradation of existing SOM (Fontaine *et al.*, 2004; Hoosbeek *et al.*, 2004) through both abiotic and biotic mechanisms (reviewed by Kuzyakov *et al.*, 2000). However, the trade-off between C priming and nutrient availability, on the one hand, and tree growth on the other remains largely unknown. Under elevated CO_2, exudation is thought to be increased on a per-plant basis via stimulation of root growth, although experimental evidence is still lacking.

Taken together, C transferred from trees to root symbionts and rhizodeposition account for a significant proportion of net C assimilation. The tree benefits from this C loss in terms of microbial activity and the consequent nutrient acquisition and cycling through the turnover of SOM. These complex interactions between the tree and the soil suggest that C availability is not the primary resource limitation for tree growth. If C were limiting tree growth, it would only be through C limitation to nutrient cycling in forest soils.

IV. The ecosystem scale: how forests respond to environmental change

The third line of evidence that tree growth is seldom limited by C comes from a consideration of the impact of global environmental change on forests. Forest growth has increased in recent decades, as shown by studies of temperate forests in North America (Turner *et al.*, 1995), Europe (Hunter & Schuck, 2002; Solberg *et al.*, 2004) and tropical forests in Amazonia (Baker *et al.*, 2004). Does this mean that the increased forest growth we now see, as atmospheric CO_2 concentrations rise, is because trees have been, and are, C-limited? There have been two different approaches used to try and explain these growth increments. Manipulative experiments (especially applying elevated CO_2 to trees) and models have been used to try to determine the combined and interactive effects of elevated CO_2, changes in climate (such as temperature and rainfall) and pollutant loadings (particularly N deposition) on forest growth and C sequestration.

1. Responses to elevated CO_2 and the importance of soil processes

Hundreds of papers have now been published describing the responses of trees to elevated CO_2, including many reviews (Ceulemans & Mousseau, 1994; Curtis & Wang, 1998; Saxe *et al.*, 1998; Gielen & Ceulemans, 2001; Long *et al.*, 2004; Nowak *et al.*, 2004; Ainsworth & Long, 2005). While many have reported that elevated CO_2 stimulates forest tree growth, some have found only a short-term growth enhancement or no stimulation of above-ground growth, but enhanced root growth, so NPP is increased. Interpreting the results from these studies is complex, as responses are confounded by the timescale of the studies, the stage of stand growth (expanding vs full canopy) and the relative availability of other resources, such as nutrients (see Körner, 2006). However, in the few studies where elevated CO_2 has been given to forest systems with a near to steady-state nutrient cycle and full canopy development, initial responses in above-ground growth have declined through time (Körner, 2006).

The scale of most elevated CO_2 experiments is not that of the forest, and even the longest duration study still only reflects a small fraction of the lifetime of a tree (Norby *et al.*, 1999). The experiments that have provided elevated CO_2 at a scale closest to that of the forest are the FACE experiments. There have been four of these: at Duke Forest, NC, USA, growing loblolly pine (from 1996, Hendrey *et al.*, 1999); at Rhinelander, WI, USA, growing aspen, sugar maple and birch (from 1997, Karnosky *et al.*, 1999); at Oak Ridge, TN, USA, growing sweetgum (from 1998, Norby *et al.*, 2001) and at the POPFACE site in Italy, growing poplars (from 1999, Miglietta *et al.*, 2001). These experiments have studied the response of plantations, which between them cover a wide range of NPP (Norby *et al.*, 2005), to an addition of an extra 200 μmol mol^{-1}

CO_2 above ambient. In addition, there has been one experiment which has used a derivation of the FACE technology (called Web-FACE) to study the response of a range of 35-m-high deciduous forest trees to a 530 μmol mol^{-1} CO_2 atmosphere over 4 yr (Körner *et al.*, 2005).

A common response to elevated CO_2 in all the tree FACE experiments has been an increase in both above-ground and below-ground production, as a result of faster photosynthetic rates (Nowak *et al.*, 2004). Despite both the AspenFACE and POPFACE experiments being initiated on bare soil, all the FACE sites will have 'primed' the system with extra C, causing a disruption so the system is no longer in equilibrium. The feedback loop (B) shown in Fig. 1 between the forest soil and the tree operates at a timescale of up to decades, while the flux of C from the tree into the soil (3) is regulated by physiological processes operating over a considerably shorter timescale. However, it is not clear if any of the experiments have yet reached a new equilibrium or stable state (Körner, 2006). Therefore, it is difficult to know if the responses seen to the abrupt change in atmospheric CO_2 concentrations arising in FACE experiments would be the same as those to the (comparatively) gradual increase predicted over the next 50 yr or so, where the C priming effect will be much less and forests remain closer to a steady state throughout the transition. A major question then is as follows: will nutrient supply rate keep pace with the demand created by faster tree growth under elevated CO_2? This has been questioned (Zak *et al.*, 2000) and it has been suggested that available soil N may increasingly limit growth as C and N are sequestered in woody biomass and SOM (Luo *et al.*, 2004). However, the actual evidence for this in FACE experiments is still sparse, with recent reviews showing little evidence at the Oak Ridge site yet (Norby & Iversen, 2006) and, while there has been immobilization of N in tree biomass and soil at Duke Forest site, ecosystem C : N ratios have increased under elevated CO_2 and there has been a large accumulation of ecosystem N capital (Finzi *et al.*, 2006).

Trees have two options for maintaining faster growth rates under elevated CO_2 if soil N availability does not keep pace with their increased demand for N. First, they can increase their N-use efficiency. This will happen as a result of the kinetic properties of Rubisco, as the oxygenase function is suppressed at higher $[CO_2]$. It can also occur through photosynthetic acclimation, reducing N lost through leaf senescence or changing patterns of C/N allocation, as discussed above. Second, they can use their extra C to acquire more N from the soil through increased allocation below ground, specifically to fine roots and their mycorrhizal fungi. One mechanism for this could be a tighter coupling between litter production and direct uptake of organic N from the litter by mycorrhizal fungi (Fig. 2). So far, the potential for mycorrhizal fungi to bypass litter decomposition by saprotrophs by direct nutrient transfer from litter to tree roots has been widely overlooked (Chapman *et al.*, 2006). Yet it could play a significant role in

meeting tree N demand under elevated CO_2, because litter production is increased (Finzi *et al.*, 2001).

To increase the nutrient capital available to trees in the longer term, SOM turnover rates would have to increase, and consequently soil respiration rates would also increase. Soil respiration has two components. The autotrophic respiration (Ra) is associated with the use of C recently assimilated by the tree, including growth and maintenance of roots and their mycorrhizal fungi and the rhizosphere microbial respiration tightly coupled to the supply of C from rhizodeposition (flux 6 in Fig. 1). Heterotrophic respiration (Rh) involves the wide range of organisms involved in the SOM decomposition food web (flux 5 in Fig. 1), possibly including respiration of extra-radical mycorrhizal network. Thus Rh relies upon 'historical' C but, through the turnover of SOM, accelerates the rate of soil nutrient cycling. Forest soil respiration rates vary considerably in relation to soil moisture and temperature (Tedeschi *et al.*, 2006). However, the biotic factors governing Rh in forest soils are not well understood. Therefore, distinguishing Ra from Rh, and particularly how the latter is regulated, is important for understanding the processes regulating SOM turnover and, ultimately, whether with rising atmospheric CO_2 concentrations nutrient cycling in the soil will be affected.

It is well established that elevated CO_2 increases soil respiration rate under a wide range of vegetation types, including trees (reviewed by Zak *et al.*, 2000) and in tree FACE experiments (King *et al.*, 2004; Bernhardt *et al.*, 2006). But is there any evidence that Rh and N cycling increases with elevated CO_2? After 6 yr of elevated CO_2 at the Duke Forest site, there was evidence of a dynamic or transient increase in the C sink in the forest floor (Lichter *et al.*, 2005), although the magnitude of the increase was limited by fast turnover times (estimated using the $\delta^{13}C$ signature of the CO_2 used to supplement the atmosphere as a tracer for the C). This was interpreted as being the result of increased fine root turnover, suggesting that Rh would be unaffected. At the Oak Ridge site, enhanced NPP led to greater fine root production (Norby *et al.*, 2004) and a slight increase in Rh (Norby *et al.*, 2002). However, no effect of elevated CO_2 was found on the microbial biomass or N cycling processes (Sinsabaugh *et al.*, 2003). In a study of soil N cycling across the three FACE experiments in the USA, Zak *et al.* (2003) found that increased amounts of root and leaf litter had no effect on microbial N cycling pools or processes. At the POPFACE site in Italy, the rate of soil respiration under the young trees was increased by elevated CO_2 (King *et al.*, 2004), but the annual production of leaf litter was not affected, while litter chemistry was, with a decrease in N concentration (Cotrufo *et al.*, 2005). As a consequence, the leaf litter showed a slightly slower decomposition rate, although the rate of degradation of control litter was also very slow, perhaps reflecting the recent land use change at the POPFACE site affecting the capacity of the soil microbial community to degrade a 'new' substrate of poplar litter (Cotrufo *et al.*, 2005). The Web-FACE experiment found a rapid transfer of C to soil

and an increase in soil CO_2 concentrations (interpreted as enhanced soil respiration), but no measurable stimulation in stem growth (Körner *et al.*, 2005). A meta-analysis of 117 FACE and open-top chamber studies showed increased soil C concentrations under elevated CO_2, but no increase in total soil N, net N mineralization or N_2 fixation (De Graaff *et al.*, 2006). In unfertilized ecosystems, microbial N immobilization was found to decrease any stimulation of plant growth to elevated CO_2 (De Graaff *et al.*, 2006).

So it is clear that there is a C-priming effect at the FACE sites, with increases in NPP (DeLucia *et al.*, 2005), but there is not yet evidence that the system has reached a new steady state with respect to the interaction between soil C and N cycling in any of the experiments. While soil respiration has been increased by FACE, at some sites (with established forest) the stimulation in rate has declined through time (King *et al.*, 2004; Bernhardt *et al.*, 2006) and there is little evidence that Rh has been affected or soil N cycling altered. One possible reason for the contradictory results from FACE experiments of enhanced tree N uptake and even soil N depletion, without measurable changes in soil N mineralization, is that our measures of mineralization and soil N availability are not sensitive enough (Johnson, 2006), or are addressing a process of minor relevance for assessing nutrient availability to trees on infertile soils (Schimel & Bennett, 2004). However, taken together, these results suggest that, in the medium term, N cycling is unlikely to keep pace with the increases in NPP caused by C priming, resulting in an overall nutrient limitation rather than C limitation to forest growth.

2. Ecosystem modelling

Turning now to consider modelling, several studies have used large forest inventory datasets along with climate and other environmental data to determine the causes of observed growth changes in forests (Nellemann & Thomsen, 2001; Ollinger *et al.*, 2002; Solberg *et al.*, 2004). The advantage of such approaches is that multiple factors can be accounted for, including changing weather patterns and N inputs from atmospheric deposition. The modelling has suggested that in Europe the majority of forest growth increment can be accounted for by N deposition (Nellemann & Thomsen, 2001; Solberg *et al.*, 2004) and very little by elevated CO_2. In North America the picture is further complicated, with ozone pollution in the USA potentially offsetting much of the gain attributable to either elevated CO_2 or N deposition (Ollinger *et al.*, 2002). However, an analysis of tree ring chronologies has suggested that atmospheric CO_2 increases since 1950 have increased the growth of ponderosa pine growing on water-limited sites in the Pacific Northwest (Soulé & Knapp, 2006). A similar approach, comparing tree rings in individuals growing near a natural CO_2 vent with control trees at ambient CO_2, demonstrated after 30 yr a 12% growth response, found mainly when the trees were young and in years that had a dry

spring (Hättenschwiler *et al.*, 1997). In boreal Canada, disturbances caused by fire or insect attack appear to have resulted in an overall reduction in forest growth in recent years (Kurz & Apps, 1999). There have been fewer studies of tropical forests, although it has recently been suggested that their response will be highly dependent upon factors such as increased rainfall patterns, which are predicted to reduce growth (Schuur, 2003) and could be interpreted as a potential nutrient limitation as a result of increased leaching.

In addition to these large-scale modelling studies, models of forest growth have been used to simulate the long-term effects of elevated CO_2 on forest growth and plant–soil C dynamics (Medlyn *et al.*, 2000; McMurtrie *et al.*, 2001). Such studies have questioned the relevance of short-term experimentation in systems where longer-term biogeochemical feedbacks operate (Kirschbaum *et al.*, 1994; Rastetter *et al.*, 1997). In particular, the importance of soil processes (especially N immobilization and mineralization) in regulating long-term responses to elevated CO_2 has been highlighted (Medlyn *et al.*, 2000; McMurtrie *et al.*, 2001). These ecosystem simulation models suggest a down-regulation of NPP in response to an increase in atmospheric CO_2, when plant uptake of N exceeds the rate of replenishment via mineralization (Rastetter *et al.*, 1997; Luo & Reynolds, 1999). With a model calibrated to an old growth forest in the north-eastern United States, Rastetter *et al.* (1997) identified this as happening on a timescale of several decades, which is significantly longer than the duration of any of the FACE experiments.

V. Conclusions

It should be clear from the arguments presented that, at each of the scales considered, there is little evidence for a direct C limitation of tree growth. While no one piece of evidence is conclusive, taken together the weight of evidence suggests that the physiological functioning and growth of individual trees is not limited by the availability of C. Evidence from both the biochemical (leaf) and physiological (whole tree) scales suggests that forest trees have an abundance of C compared with nutrients such as N or P. However, given what is known about the complex interactions between the C and nutrient dynamics of forest ecosystems, discussed earlier, it is tempting to think that the overall rates of SOM turnover and biogeochemical cycling of nutrients are limited by the inputs of C from vegetation.

So if tree growth is not C-limited, is their nutrient supply? Does C cycling interact with the N cycle to impose an overall limitation on tree growth? Based on current evidence, the answer to this is 'probably not'. Where FACE experiments have provided a C priming to a forest system, there has been a faster cycling of C through the trees to the soil and back to the atmosphere because of increased soil autotrophic respiration. But the lack of evidence for an impact on N cycling processes suggest that all this extra labile C entering the soil

has little impact on nutrient supply through SOM turnover. This suggests that the faster tree growth that has been observed with FACE has been supported by increased N use efficiency and N cycling, possibly through the interactions of mycorrhizal fungi and leaf litter.

Although carbon assimilation increases with increasing atmospheric CO_2, it does so at a diminishing rate. One reason for this could be a feedback effect resulting from an eventual sink limitation on photosynthesis, once the rate of autotrophic respiration has reached a new steady state (determined perhaps by a nutrient limitation). Second, respiration is an exponentially increasing function of temperature. Thus, as global change proceeds, the rate of increase of CO_2 assimilation by terrestrial ecosystems will slow, while rates of soil respiration will increase. One possible feedback might be that, if the turnover of SOM increases, this could increase nutrient cycling and so enhance soil fertility. In the short term, there will be a positive effect on growth and therefore on CO_2 uptake, but over longer time frames (decades), the net effect is likely to be a decrease in the ability of the terrestrial biosphere to absorb CO_2.

Acknowledgements

References

Adams F, Reddell P, Webb MJ, Shipton WA. 2006. Arbuscular mycorrhizas and ectomycorrhizas on *Eucalyptus grandis* (Myrtaceae) trees and seedlings in native forests of tropical north-eastern Australia. *Australian Journal of Botany* **54**: 271–281.

Aerts R. 1996. Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology* **84**: 597–608.

Aerts R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. *Journal of Experimental Botany* **50**: 29–37.

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

Alberton O, Kuyper TW, Gorissen A. 2005. Taking mycocentrism seriously: mycorrhizal fungal, plant responses to elevated CO_2. *New Phytologist* **167**: 859–868.

Alexander IJ. 2006. Ectomycorrhizas – out of Africa? *New Phytologist* **172**: 589–591.

Allison SD, Vitousek PM. 2004. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. *Biotropica* **36**: 285–296.

Ameglio T, Decourteix M, Alves G, Valentin V, Sakr S, Juliene JL, Pétel G, Guilliot A, Lacointe A. 2004. Temperature effects on xylem sap osmolarity in walnut trees: evidence for a vitalistic model of winter embolism repair. *Tree Physiology* **24**: 785–793.

Andersson I, Taylor TC. 2003. Structural framework for catalysis and regulation in ribulose-1,5-bisphosphate carboxylase/oxygenase. *Archives of Biochemistry and Biophysics* **414**: 130–140.

Bainbridge G, Madgwick P, Parmar S, Mitchell R, Paul M, Pitts J, Keys AJ, Parry MAJ. 1995. Engineering Rubisco to change its catalytic properties. *Journal of Experimental Botany* **46**: 1269–1276.

Baker TR, Phillips OL, Malhi Y, Almeida S, Arroyo L, Di Fiore A, Erwin T, Hiquchi N, Killeen TJ, Laurance SG, Laurance WF, Lewis SL, Monteaqudo A, Neill DA, Vargas PN, Pitman NCA, Silva JNM, Martinez RV. 2004. Increasing biomass in Amazonian forest plots. *Philosophical Transactions of the Royal Society of London Series B-Biology Sciences* **359**: 353–365.

Bernhardt ES, Barber JJ, Pippen JS, Taneva L, Andrews JA, Schlesinger WH. 2006. Long-term effects of free air CO$_2$ enrichment (FACE) on soil respiration. *Biogeochemistry* **77**: 91–116.

Bhupinderpal-Singh, Nordgren A, Lofvenius MO, Höbgerg MN, Mellander PE, Högberg P. 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant, Cell & Environment* **26**: 1287–1296.

Binkley D, Stape JL, Takahashi EN, Ryan MG. 2006. Tree-girdling to separate root, heterotrophic respiration in two Eucalyptus stands in Brazil. *Oecologia* **148**: 447–454.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics* **16**: 363–392.

Brandes B, Godbold DL, Kuhn AJ, Jentschke G. 1998. Nitrogen and phosphorus acquisition by the mycelium of the ectomycorrhizal fungus *Paxillus involutus* and its effect on host nutrition. *New Phytologist* **140**: 735–743.

van Breemen N, Findlay R, Lundström U, Jongmans AG, Giesler R, Olsson M. 2000. Mycorrhizal weathering: a true case of mineral plant nutrition? *Biogeochemistry* **49**: 53–67.

von Caemmerer S, Furbank RT. 2003. The C-4 pathway: an efficient CO$_2$ pump. *Photosynthesis Research* **77**: 191–207.

Camm E. 1993. Photosynthetic responses in developing and year-old Douglas-fir needles during new shoot development. *Trees – Structure and Function* **8**: 61–66.

Carswell FE, Millard P, Rogers GND, Whitehead D. 2003. Influence of nitrogen and phosphorus supply on foliage growth and internal recycling of nitrogen in conifer seedlings (*Prumnopitys ferruginea*). *Functional Plant Biology* **30**: 49–55.

Catt JW, Millard P. 1988. The measurement of Ribulose 1, 5-*bis*phosphate carboxylase/ oxygenase concentration in the leaves of potato plants by enzyme linked immunosorbtion assays. *Journal of Experimental Botany* **39**: 157–164.

Ceulemans R, Mousseau M. 1994. Effects of elevated atmospheric CO$_2$ on woody-plants. *New Phytologist* **127**: 425–446.

Chadwick O, Derry LA, Vitousek PM, Huebert BJ, Hedin LO. 1999. Changing sources of nutrients during four million years of ecosystem development. *Nature* **397**: 491–497.

Chapin FS. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**: 233–260.

Chapin FS, Bloom AJ, Field CB. 1987. Plant responses to multiple environmental factors. *Bioscience* **37**: 49–57.

Chapin FS, Shaver GR. 1985. Individualistic growth response of tundra plant species to environmental manipulations in the field. *Ecology* **66**: 564–576.

Chapman SK, Langley JA, Hart SC, Koch GW. 2006. Plants actively control nitrogen cycling: uncorking the microbial bottleneck. *New Phytologist* **169**: 27–34.

Cheng L, Fuchigami L. 2000. Rubisco activation state decreases with increasing nitrogen content in apple leaves. *Journal of Experimental Botany* **51**: 1687–1694.

Cooke JEK, Weih M. 2005. Nitrogen storage and seasonal nitrogen cycling in *Populus*: bridging molecular physiology and ecophysiology. *New Phytologist* **167**: 19–30.

Cornelissen JH, Perez-Harguindeguy N, Diaz S, Grime JP, Marzano B, Cabido M, Vendramini F, Cerabolini B. 1999. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytologist* **143**: 191–200.

Cotrufo MF, De Angelis P, Polle A. 2005. Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO$_2$ enrichment (POPFACE). *Global Change Biology* **11**: 971–982.

Crafts-Brander SJ, Salvucci ME, Egli DB. 1990. Changes in ribulose biphosphate carboxylase/oxygenase and ribulose 5-phosphate kinase abundances and photosynthetic capacity during leaf senescence. *Photosynthetic Research* **23**: 223–230.

Curtis PS, Wang XZ. 1998. A meta-analysis of elevated CO$_2$ effects on woody plant mass, form, and physiology. *Oecologia* **113**: 299–313.

De Graaff M-A, Van Groenigen K-J, Six J, Hungate B, van Kessel C. 2006. Interactions between plant growth and soil nutrient cycling under elevated CO$_2$: a meta-analysis. *Global Change Biology* **12**: 2077–2091.

Del Arco JM, Escudero A, Garrido MV. 1991. Effects of site characteristics on nitrogen retranslocation from senescing leaves. *Ecology* **72**: 701–708.

DeLucia EH, Moore DJ, Norby RJ. 2005. Contrasting responses of forest ecosystems to rising atmospheric CO$_2$: Implications for the global C cycle. *Global Biogeochemical Cycles* **19**: GB3006.

Dodd AN, Borland AM, Haslam RP, Griffiths H, Maxwell K. 2002. Crassulacean acid metabolism: plastic, fantastic. *Journal of Experimental Botany* **53**: 569–580.

Drake BG, Gonzalez-Meler MA, Long SP. 1997. More efficient plants: a consequence of rising atmospheric CO$_2$? *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 609–639.

Dyckmans J, Flessa H. 2001. Influence of tree internal N status on uptake and translocation of C and N in beech: a dual ^{13}C and ^{15}N labeling approach. *Tree Physiology* **21**: 395–401.

Egerton-Warburton L, Allen MF. 2001. Endo-, ectomycorrhizas in *Quercus agrifolia* Nee. (Fagaceae): patterns of root colonization, effects on seedling growth. *Mycorrhiza* **11**: 283–290.

Ehleringer JR, Cerling TE, Helliker BR. 1997. C$_4$ photosynthesis, atmospheric CO$_2$, and climate. *Oecologia* **112**: 285–299.

Fahey TJ, Tierney GL, Fitzhugh RD, Wilson GF, Siccama TG. 2005. Soil respiration and soil carbon balance in a northern hardwood forest ecosystem. *Canadian Journal of Forest Research* **35**: 244–253.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO$_2$ assimilation in leaves of C-3 species. *Planta* **149**: 78–90.

Farrar J, Hawes M, Jones D, Lindow S. 2003. How roots control the flux of carbon to the rhizosphere. *Ecology* **84**: 827–837.

Finzi AC, Allen S, DeLucia EH, Ellsworth DF, Schlesinger WH. 2001. Forest litter production, chemistry, and decomposition following two years of free-air CO$_2$ enrichment. *Ecology* **82**: 470–484.

Finzi AC, Moore DJP, DeLucia EH, Lichter J, Hofmockel KS, Jackson RB, Kim HS, Matamala R, McCarthy HR, Oren R, Pippen JS, Schlesinger WH. 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO$_2$ in a warm-temperate forest. *Ecology* **87**: 15–25.

Fogel R, Hunt G. 1983. Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. *Canadian Journal of Forest Research* **13**: 219–232.

Fontaine S, Bardoux G, Abbadie L, Mariotti A. 2004. Carbon input to soil may decrease soil carbon content. *Ecology Letters* **7**: 314–320.

Fransson PMA, Taylor AFS, Finlay RD. 2001. Elevated atmospheric CO$_2$ alters root symbiont community structure in forest trees. *New Phytologist* **152**: 431–442.

Gansert D, Sprick W. 1998. Storage and mobilization of nonstructural carbohydrates and biomass development of beech seedlings (*Fagus sylvatica* L.) under different light regimes. *Trees-Structure and Function* **12**: 247–257.

Gehring CA, Connell JH. 2006. Arbuscular mycorrhizal fungi in the tree seedlings of two Australian rain forests: occurrence, colonization and relationships with plant performance. *Mycorrhiza* **6**: 89–98.

Giardina C, Coleman M, Hancock J, King J, Lilleskov E, Loya W, Pregitzer K, Ryan M, Trettin C. 2005. The effects of global change on belowground carbon allocation in forests. In: Binkley D, Menyailo O, eds. *The impacts of global climate change on plant – soil interactions.* NATO Science Series. Dordrecht, the Netherlands: Kluwer Academic Press, Ch 7.

Giardina CP, Ryan MG, Binkley D, Fownes JH. 2003. Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. *Global Change Biology* **9**: 1438–1450.

Gielen B, Ceulemans R. 2001. The likely impact of rising atmospheric CO_2 on natural and managed *Populus*: a literature review. *Environmental Pollution* **115**: 335–358.

Gleason SM, Ares A. 2004. Photosynthesis, carbohydrate storage and survival of a native and an introduced tree species in relation to light and defoliation. *Tree Physiology* **24**: 1087–1097.

Godbold DL, Hoosbeek MR, Lukac M, Cotrufo MF, Janssens IA, Ceulemans R, Polle A, Velthorst EJ, 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 and Soil* **281**: 15–24.

Graham JH, Eissenstat DM. 1998. Field evidence for the carbon cost of citrus mycorrhizas. *New Phytologist* **140**: 103–110.

Grayston SJ, Vaughan D, Jones D. 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology* **5**: 29–56.

Griffin KL, Tissue DT, Turnbull MH, Whitehead D. 2000. The onset of photosynthetic acclimation to elevated CO_2 partial pressure in field-grown *Pinus radiata* D. Don. after four years. *Plant, Cell & Environment* **23**: 1089–1098.

Hättenschwiler S, Miglietta F, Raschi F, Körner C. 1997. Thirty years of *in situ* tree growth under elevated CO_2: a model for future forest responses? *Global Change Biology* **3**: 463–471.

Hendrey GR, Ellsworth DS, Lewin KF, Nagy J. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology* **5**: 293–309.

Hessen DO, Ågren GI, Anderson TR, Elser JJ, De Ruiter PC. 2004. Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology* **85**: 1179–1192.

Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil* **237**: 173–195.

Hoch G, Körner C. 2003. The carbon charging of pines at the climatic treeline; a global comparison. *Oecologia* **135**: 10–21.

Hoch G, Richter A, Körner C. 2003a. Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment* **26**: 1067–1081.

Hoch WA, Singsaas EL, McCown BH. 2003b. Resorption protection. Anthocyanins facilitate nutrient recovery in autumn by shielding leaves from potentially damaging light levels. *Plant Physiology* **133**: 1296–1305.

Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**: 297–299.

Högberg MN, Högberg P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist* **154**: 791–795.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* **411**: 789–792.

Hoosbeek MR, Lukac M, van Dam D, Godbold DL, Velthorst EJ, Biondi FA, Peressotti A, Cortufo 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? *Global Biogeochemical Cycles* **18**: art–GB1040.

Hunter I, Schuck A. 2002. Increasing forest growth in Europe – possible causes and implications for sustainable forest management. *Plant Biosystems* **136**: 133–141.

Johnson DW. 2006. Progressive N limitation in forests: review and implications for long-term responses to elevated CO_2. *Ecology* **87**: 64–75.

Johnson D, Ijdo M, Genney DR, Anderson IC, Alexander IJ. 2005a. How do plants regulate the function, community structure, and diversity of mycorrhizal fungi? *Journal of Experimental Botany* **56**: 1751–1760.

Johnson D, Krsek M, Wellington EMH, Stott AW, Cole L, Bardgett RD, Read DJ, Leake JR. 2005b. Soil invertebrates disrupt carbon flow through fungal networks. *Science* **309**: 1047–1047.

Johnson D, Leake JR, Read DJ. 2002. Transfer of recent photosynthate into mycorrhizal mycelium of an upland grassland: short-term respiratory losses and accumulation of ^{14}C. *Soil Biology and Biochemistry* **34**: 1521–1524.

Jones DL, Hodge A, Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* **163**: 459–480.

Jordan MO, Habib R. 1996. Mobilizable carbon reserves in young peach trees as evidenced by trunk girdling experiments. *Journal of Experimental Botany* **47**: 79–87.

Kagawa A, Sugimoto A, Maximov TC. 2006a. Seasonal course of translocation, storage and remobilization of ^{13}C pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings. *New Phytologist* **171**: 793–804.

Kagawa A, Sugimoto A, Maximov TC. 2006b. $^{13}CO_2$ pulse-labelling of photoassimilates reveals carbon allocation within and between tree rings. *Plant, Cell & Environment* **29**: 1571–1584.

Kang S-M, Titus JS. 1980. Qualitative and quantitative changes in nitrogen compounds in senescing leaf and bark tissue of the apple. *Physiolgia Plantarum* **50**: 285–290.

Karnosky DF, Mankovska B, Percy K, Dickson RE, Podila GK, Sober J, Noormets A, Hendrey G, Coleman MD, Kubiske M, Pregitzer KS, Isebrands JG. 1999. Effects of tropospheric O_3 on trembling aspen and interaction with CO_2: Results from an O_3-gradient and a face experiment. *Water Air and Soil Pollution* **116**: 311–322.

King JS, Hanson PJ, Bernhardt E, DeAngelis P, Norby RJ, Prequitzer KS. 2004. A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments. *Global Change Biology* **10**: 1027–1042.

Kirschbaum MUF, King DA, Comins HN, McMurtrie RD, Medlyn BE, Pongracic S, Murty D, Keith H, Raison RJ, Khanna PK, Sheriff DW. 1994. Modelling forest response to increasing CO_2 concentration under nutrient limited conditions. *Plant, Cell & Environment* **17**: 1081–1099.

Kobe RK. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos* **80**: 226–233.

Körner C. 2003. Carbon limitation in trees. *Journal of Ecology* **91**: 4–17.

Körner C. 2006. Plant CO_2 responses: an issue of definition, time and resource supply. *New Phytologist* **172**: 393–411.

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

Kurz WA, Apps MJ. 1999. A 70-year retrospective analysis of carbon fluxes in the Canadian forest sector. *Ecological Applications* **9**: 526–547.

Kuzyakov Y, Friedel JK, Stahr K. 2000. Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* **32**: 1485–1498.

Langley JA, Chapman SK, Hungate BA. 2006. Ectomycorrhizal colonization slows root decomposition: the *post-mortem* fungal legacy. *Ecology Letters* **9**: 955–959.

Langley JA, Hungate BA. 2003. Mycorrhizal controls on belowground litter quality. *Ecology* **84**: 2302–2312.

Lichter J, Barron SH, Bevacqua CE, Finzli. C, Irving KE, Stemmler EA, Schlesinger WH. 2005. Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO_2 enrichment. *Ecology* **86**: 1835–1847.

von Liebig J,. 1840. *Die organische Chemie in Ihrer Anwendung auf Agricultur und Physiologie.* Braunschweig, Germany: Friedrich Vieweg und Sohn.

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist* **173**: 611–620.

Lindahl BO, Taylor AFS, Finlay RD. 2002. Defining nutritional constraints on carbon cycling in boreal forests – towards a less 'phytocentric' perspective. *Plant and Soil* **242**: 123–135.

Loewe A, Einig W, Shi L, Dizengreme P, Hampp R. 2000. Mycorrhiza formation and elevated CO_2 both increase the capacity for sucrose synthesis in source leaves of spruce and aspen. *New Phytologist* **145**: 565–574.

Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: plants face the future. *Annual Review of Plant Biology* **55**: 591–628.

Lovelock CE, Kyllo D, Popp M, Isopp H, Virgo A, Winter K. 1997. Symbiotic vesicular-arbuscular mycorrhizae influence maximum rates of photosynthesis in tropical tree seedlings grown under elevated CO_2. *Australian Journal of Plant Physiology* **24**: 185–194.

Luo Y, Reynolds JF. 1999. Validity of extrapolating field CO_2 experiments to predict carbon sequestration in natural ecosystems. *Ecology* **80**: 1568–1583.

Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience* **54**: 731–739.

Lüttge U. 2004. Ecophysiology of crassulacean acid metabolism (CAM). *Annals of Botany* **93**: 629–652.

Lüttge U. 2006. Photosynthetic flexibility and ecophysiological plasticity: questions and lessons from *Clusia*, the only CAM tree, in the neotropics. *New Phytologist* **171**: 7–25.

Lynch JM, Whipps JM. 1990. Substrate flow in the rhizosphere. *Plant and Soil* **129**: 1–10.

Manter DK, Kavanagh KL, Rose CL. 2005. Growth response of Douglas-fir seedlings to nitrogen fertilization: importance of Rubisco activation state and respiration rates. *Tree Physiology* **25**: 1015–1021.

Maurel K, Leite GB, Bonhomme M, Guilliot A, Rageau R, Pétel G, Sakr S. 2004. Trophic control of bud break in peach (*Prunus persica*) trees: a possible role of hexoses. *Tree Physiology* **24**: 579–588.

McMurtrie RE, Medlyn BE, Dewar RC. 2001. Increased understanding of nutrient immobilization in soil organic matter is critical for predicting the carbon sink strength of forest ecosystems over the next 100 years. *Tree Physiology* **21**: 831–839.

Medlyn BE, Badeck F-W, De Pury DGG, Barton CVM, Broadmeadow M, Ceulemans R, De Angelis P, Forstreuter M, Jach ME, Kellomäki S, Laitat E, Marek M, Philippot S, Rey A, Strassemeyer J, Laitinen K, Liozon R, Portier B, Roberntz P, Wang K et al. 1999. Effects of elevated [CO_2] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant, Cell & Environment* **22**: 1475–1495.

Medlyn BE, McMurtrie RE, Dewar RC, Jeffreys MP. 2000. Soil processes dominate the long – term response of forest net primary productivity to increased temperature and atmospheric CO_2 concentration. *Canadian Journal of Forest Research* **30**: 873–888.

Melillo JM, Mcguire AD, Kicklighter DW, Moore B, Vorosmarty CJ, Schloss AL. 1993. Global climate-change and terrestrial net primary production. *Nature* **363**: 234–240.

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

Millard P. 1988. The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell & Environment* **11**: 1–8.

Millard P. 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. *Zeitschrift für Pflanzenernahrung und Bodenkunde* **159**: 1–10.

Millard P, Catt JW. 1988. The influence of nitrogen supply on the use of nitrate and ribulose 1,5-bisphosphate carboxylase/oxygenase as leaf nitrogen stores for growth of potato tubers (*Solanum tuberosum* L.). *Journal of Experimental Botany* **39**: 1–11.

Millard P, Hester A, Wendler R, Baillie G. 2001. Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Functional Ecology* **15**: 535–543.

Millard P, Proe MF. 1993. Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bong) Carr as influenced by nitrogen supply. *New Phytologist* **125**: 113–119.

Millard P, Thomson CM. 1989. The effect of the autumn senescence of leaves on the internal cycling of nitrogen for the spring growth of apple-trees. *Journal of Experimental Botany* **40**: 1285–1289.

Moyersoen B, Fitter AH, Alexander IJ. 1998. Spatial distribution of ectomycorrhizas, arbuscular mycorrhizas in Korup National Park rain forest, Cameroon, in relation to edaphic parameters. *New Phytologist* **139**: 311–320.

Näsholm T. 1994. Removal of nitrogen during needle senescence in Scots pine (*Pinus sylvestris* L.). *Oecologia* **99**: 290–296.

Neftel A, Oeschger H, Staffelbach T, Stauffer B. 1988. CO_2 record in the Byrd ice core 50 000–5000 years Bp. *Nature* **331**: 609–611.

Nellemann C, Thomsen MG. 2001. Long-term changes in forest growth: Potential effects of nitrogen deposition and acidification. *Water Air and Soil Pollution* **128**: 197–205.

Neumann G, Römheld V. 2001. The release of root exudates as affected by the plant's physiological status. In: Pinton R, Varanini Z, Nannipieri P, eds. *The rhizosphere.* New-York, USA: Marcel Dekker Inc, 41–94.

Neville J, Tessier JL, Morrison I, Scarratt J, Canning B, Klironomos JN. 2002. Soil depth distribution of ecto- and arbuscular mycorrhizal fungi associated with *Populus tremuloides* within a 3-year-old boreal forest clear-cut. *Applied Soil Ecology* **19**: 209–216.

Niinemets Ü, Tamm Ü. 2005. Species differences in timing of leaf fall and foliage chemistry modify nutrient resorption efficiency in deciduous temperate forest stands. *Tree Physiology* **25**: 1001–1014.

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

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

Norby RJ, Iversen CM. 2006. Nitrogen uptake, distribution, turnover, and efficiency of use in a CO_2-enriched sweetgum forest. *Ecology* **87**: 5–14.

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG. 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. *Proceedings of the National Academy of Sciences, USA* **101**: 9689–9693.

Norby RJ, Todd DE, Fults J, Johnson DW. 2001. Allometric determination of tree growth in a CO_2-enriched sweetgum stand. *New Phytologist* **150**: 477–487.

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R. 1999. Tree responses to rising CO_2 in field experiments: implications for the future forest. *Plant, Cell & Environment* **22**: 683–714.

Nowak RS, Ellsworth DS, Smith SD. 2004. Functional responses of plants to elevated atmospheric CO_2 – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162**: 253–280.

Ollinger SV, Aber JD, Reich PB, Freuder RJ. 2002. Interactive effects of nitrogen deposition, tropospheric ozone, elevated CO_2 and land use history on the carbon dynamics of northern hardwood forests. *Global Change Biology* **8**: 545–562.

Ono K, Nishi Y, Watanabe A, Terashima I. 2001. Possible mechanisms of adaptive leaf senescence. *Plant Biology* **3**: 234–243.

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

Parrent JL, Morris WF, Vigalys R. 2006. CO_2 – enrichment and nutrient availability alter ectomycorrhizal fungal communities. *Ecology* **87**: 2278–2287.

Parry MAJ, Andralojc PJ, Mitchell RAC, Madgwick PJ, Keys AJ. 2003. Manipulation of Rubisco: the amount, activity, function and regulation. *Journal of Experimental Botany* **54**: 1321–1333.

Pearson PN, Palmer MR. 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature* **406**: 695–699.

Phillips RP, Fahey TJ. 2006. Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. *Ecology* **87**: 1302–1313.

Pourtau N, Mares M, Purdy S, Quentin N, Ruel A, Wingler A. 2004. Interactions of abscisic acid and sugar signalling in the regulation of leaf senescence. *Planta* **219**: 765–772.

Prescott CE. 2002. The influence of the forest canopy on nutrient cycling. *Tree Physiology* **22**: 1193–1200.

Quick WP, Fichtner K, Schulze ED, Wendler R, Leegood RC, Mooney H, Rodermel SR, Bogorad L, Stitt M. 1992. Decreased Ribulose–1, 5–bisphosphate carboxylase–oxygenase in transgenic tobacco transformed with an antisense *RbcS*. 4. Impact on photosynthesis in conditions of altered nitrogen supply. *Planta* **188**: 522–531.

Rastetter EB, Ågren GI, Shaver GR. 1997. Responses of N – limited ecosystems to increased CO_2: a balanced – nutrition, coupled – element – cycles model. *Ecological Applications* **7**: 444–460.

Rastetter EB, Shaver GR. 1992. A model of multiple-element limitation for acclimating vegetation. *Ecology* **73**: 1157–1174.

Read DJ. 1991. Mycorrhizas in ecosystems. *Experientia* **47**: 376–391.

Read DJ, Perez-Moreno J. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* **157**: 475–492.

Richardson SJ, Peltzer DA, Allen RB, McGlone MS, Parfitt RL. 2004. Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia* **139**: 267–276.

Rillig MC, Mummey DL. 2006. Mycorrhizas and soil structure. *New Phytologist* **171**: 41–53.

Roberntz P, Stockfors J. 1998. Effects of elevated CO_2 concentration and nutrition on net photosynthesis, stomatal conductance and needle respiration of field-grown Norway spruce trees. *Tree Physiology* **18**: 233–241.

Rogers A, Ellsworth DS. 2002. Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO_2 (FACE). *Plant, Cell & Environment* **25**: 851–858.

Rogers A, Humphries SW. 2000. A mechanistic evaluation of photosynthetic acclimation at elevated CO_2. *Global Change Biology* **6**: 1005–1011.

Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornsson B, Allen ME, Maurer GE. 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. *Ecological Monographs* **73**: 643–662.

Sage RF. 2002. Variation in the K_{cat} of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature. *Journal of Experimental Botany* **53**: 609–620.

Sage RF. 2004. The evolution of C_4 photosynthesis. *New Phytologist* **161**: 341–370.

Saxe H, Ellsworth DS, Heath J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. *New Phytologist* **139**: 395–436.

Schimel JP, Bennett J. 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* **85**: 591–602.

Schuur EAG. 2003. Productivity and global climate revisited: The sensitivity of tropical forest growth to precipitation. *Ecology* **84**: 1165–1170.

Scott-Denton LE, Rosenstiel TD, Monson RK. 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology* **12**: 205–216.

Selosse MA, Richard F, He XH, Simard SW. 2006. Mycorrhizal networks: des liaisons dangereuses? *Trends in Ecology and Evolution* **21**: 621–628.

Sinsabaugh RL, Saiya-Corka K, Long T, Osgood MP, Neher DA, Zak DR, Norby RJ. 2003. Soil microbial activity in a Liquidambar plantation unresponsive to CO_2-driven increases in primary production. *Applied Soil Ecology* **24**: 263–271.

Soderström B. 2002. Challenges for mycorrhizal research into the new millennium. *Plant and Soil* **244**: 1–7.

Solberg S, Andreassen K, Clarke N, Tørseth K, Tveito OE, Strand GH, Tornter S. 2004. The possible influence of nitrogen and acid deposition on forest growth in Norway. *Forest Ecology and Management* **192**: 241–249.

Soulé PT, Knapp PA. 2006. Radial growth rate increases in naturally occurring ponderosa pine trees: a late-20th century CO_2 fertilization effect? *New Phytologist* **171**: 379–390.

Staddon PL. 2005. Mycorrhizal fungi and environmental change: the need for a mycocentric approach. *New Phytologist* **167**: 635–637.

Staddon PL, Ramsey CB, Ostle N, Ineson P, Fitter AH. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of C-14. *Science* **300**: 1138–1140.

Stephens DW, Millard P, Turnbull MH, Whitehead D. 2001. The influence of nitrogen supply on growth and internal recycling of nitrogen in young *Nothofagus fusca* trees. *Australian Journal of Plant Physiology* **28**: 249–255.

Stitt M, Krapp A. 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment* **22**: 583–621.

Subke J-A, Hahn V, Battipaglia G, Linder S, Buchmann N, Cotrufo MF. 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia* **139**: 551–559.

Suzuki Y, Makino A, Mae T. 2001. Changes in the turnover of Rubisco and levels of mRNAs of rbcL and rbcS in rice leaves from emergence to senescence. *Plant, Cell & Environment* **24**: 1353–1360.

Tedeschi V, Rey A, Manca G, Valentín R, Jarvis PG, Borghetti M. 2006. Soil respiration in a Mediterranean oak forest at different developmental stages after coppicing. *Global Change Biology* **12**: 110–121.

Tissue DT, Griffin KL, Turnbull MH, Whitehead D. 2001. Canopy position and needle age affect photosynthetic response in field-grown *Pinus radiata* after five years of exposure to elevated carbon dioxide partial pressure. *Tree Physiology* **21**: 915–923.

Treseder KK, Cross A. 2006. Global distributions of arbuscular mycorrhizal fungi. *Ecosystems* **9**: 305–316.

Treseder KK, Masiello CA, Lansing JL, Allen MF. 2004. Species-specific measurements of ectomycorrhizal turnover under N-fertilization: combining isotopic and genetic approaches. *Oecologia* **138**: 419–425.

Turnbull MH, Tissue DT, Griffin KL, Rogers GND, Whitehead D. 1998. Photosynthetic acclimation to long-term exposure to elevated CO_2 concentration in *Pinus radiata* D. Don. is related to age of needles. *Plant, Cell & Environment* **21**: 1019–1028.

Turner DP, Koerper GJ, Harmon ME, Lee JJ. 1995. A carbon budget for forests of the conterminous United-States. *Ecological Applications* **5**: 421–436.

Van Heerwaarden LM, Toet S, Aerts R. 2003. Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. *Oikos* **101**: 664–669.

Vitousek PM, Howarth RW. 1991. Nitrogen limitation on land and in the sea – how can it occur? *Biogeochemistry* **13**: 87–115.

Wallander H. 2006. External mycorrhizal mycelia – the importance of quantification in natural ecosystems. *New Phytologist* 171: 240–242.

Wardle DA. 1992. A comparative-assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biology Reviews of the Cambridge Philosophical Society* 67: 321–358.

Warren CR. 2004. The photosynthetic limitation posed by internal conductance to CO_2 movement is increased by nutrient supply. *Journal of Experimental Botany* 55: 2313–2321.

Warren CR, Adams MA. 2001. Distribution of N, Rubisco and photosynthesis in *Pinus pinaster* and acclimation to light. *Plant, Cell & Environment* 24: 597–609.

Warren CR, Adams M. 2004. Evergreen trees do not maximize instantaneous photosynthesis. *Trends in Plant Science* 9: 270–274.

Warren CR, Adams MA, Chen Z. 2000. Is photosynthesis related to concentration of nitrogen and Rubisco in leaves of Australian native plants? *Australian Journal of Plant Physiology* 27: 407–416.

Warren CR, Dreyer E, Adams MA. 2003. Photosynthesis-Rubisco relationships in foliage of *Pinus sylvestris* in response to nitrogen supply and the proposed role of Rubisco and amino acids as nitrogen stores. *Trees-Structure and Function* 17: 359–366.

Wendler R, Carvalho PO, Pereira JS, Millard P. 1995. Role of nitrogen remobilization from old leaves for new leaf growth of *Eucalyptus globulus* seedlings. *Tree Physiology* 15: 679–683.

Wetzel S, Demmers C, Greenwood JS. 1989. Seasonally fluctuating bark proteins are a potential form of nitrogen storage in 3 temperate hardwoods. *Planta* 178: 275–281.

Wolfe DW, Gifford RM, Hilbert D, Luo YQ. 1998. Integration of photosynthetic acclimation to CO_2 at the whole-plant level. *Global Change Biology* 4: 879–893.

Wright DP, Scholes JD, Read DJ, Rolfe SA. 2000. Changes in carbon allocation and expression of carbon transporter genes in *Betula pendula* Roth. colonized by the ectomycorrhizal fungus *Paxillus involutus* (Batsch) Fr. *Plant, Cell & Environment* 23: 39–49.

Zak DR, Holmes WE, Finzi AC, Norby RJ, Schlesinger WH. 2003. Soil nitrogen cycling under elevated CO_2: a synthesis of forest face experiments. *Ecological Applications* 13: 1508–1514.

Zak DR, Pregitzer KS, King JS, Holmes WE. 2000. Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist* 147: 201–222.

Zelitch I. 1973. Plant productivity and the control of respiration. *Proceedings of the National Academy of Sciences USA* 70: 579–584.