

REVIEW

Interactions between plant growth and soil nutrient cycling under elevated CO₂: a meta-analysis

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Abstract

free air carbon dioxide enrichment (FACE) and open top chamber (OTC) studies are valuable tools for evaluating the impact of elevated atmospheric CO₂ on nutrient cycling in terrestrial ecosystems. Using meta-analytic techniques, we summarized the results of 117 studies on plant biomass production, soil organic matter dynamics and biological N₂ fixation in FACE and OTC experiments. The objective of the analysis was to determine whether elevated CO₂ alters nutrient cycling between plants and soil and if so, what the implications are for soil carbon (C) sequestration. Elevated CO₂ stimulated gross N immobilization by 22%, whereas gross and net N mineralization rates remained unaffected. In addition, the soil C:N ratio and microbial N contents increased under elevated CO₂ by 3.8% and 5.8%, respectively. Microbial C contents and soil respiration increased by 7.1% and 17.7%, respectively. Despite the stimulation of microbial activity, soil C input still caused soil C contents to increase by 1.2% yr⁻¹. Namely, elevated CO₂ stimulated overall above- and belowground plant biomass by 21.5% and 28.3%, respectively, thereby outweighing the increase in CO₂ respiration. In addition, when comparing experiments under both low and high N availability, soil C contents (+2.2% yr⁻¹) and above- and belowground plant growth (+20.1% and +33.7%) only increased under elevated CO₂ in experiments receiving the high N treatments. Under low N availability, above- and belowground plant growth increased by only 8.8% and 14.6%, and soil C contents did not increase. Nitrogen fixation was stimulated by elevated CO₂ only when additional nutrients were supplied. These results suggest that the main driver of soil C sequestration is soil C input through plant growth, which is strongly controlled by nutrient availability. In unfertilized ecosystems, microbial N immobilization enhances acclimation of plant growth to elevated CO₂ in the long-term. Therefore, increased soil C input and soil C sequestration under elevated CO₂ can only be sustained in the long-term when additional nutrients are supplied.

Nomenclature

FACE = free air carbon dioxide enrichment;
OTC = open top chamber;
SOM = soil organic matter;
SOC = soil organic carbon

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Introduction

The atmospheric CO₂ concentration has increased from 280 µmol mol⁻¹ in preindustrial times to the current level of 365 µmol mol⁻¹ and it is expected to exceed

700 $\mu\text{mol mol}^{-1}$ by the end of this century (Houghton & Ding, 2001). Elevated atmospheric CO_2 directly affects ecosystems by stimulating plant growth (Kimball & Idso, 1983; Drake *et al.*, 1997; Ainsworth & Long, 2005). Gifford (1994) suggested that increased C assimilation by plants and its subsequent sequestration in the soil may counterbalance CO_2 emissions. However, enhanced C sequestration under rising levels of CO_2 can only occur if increases in soil C input are sustained (Taylor & Lloyd, 1992; Friedlingstein *et al.*, 1995; Kicklighter *et al.*, 1999) and soil C mineralization lags behind the increase in soil C input (Raich & Schlesinger, 1992).

During the last 25 years, soil organic matter (SOM) models have been used as tools for evaluating the impact of global change on ecosystems (Jenkinson & Rayner, 1977; Paul & van Veen, 1978; van Veen & Paul, 1981; Parton *et al.*, 1992). Such models split SOM into an active, slow and passive pool, with a turnover time of 1.5, 25 and 1000 years, respectively (Parton *et al.*, 1987). They have been linked to climate models and predict that any losses in soil C due to a rising temperature will be offset by an increase in C sequestration resulting from increased atmospheric CO_2 levels (Hall *et al.*, 2000).

However, results on soil C sequestration have been inconsistent, with studies showing an increase (Rice *et al.*, 1994), no change (van Kessel *et al.*, 2006), or even a decrease (Calabritto *et al.*, 2002; Cardon *et al.*, 2002; Hoosbeek *et al.*, 2004) in soil C contents under elevated CO_2 . To explain these conflicting results, many studies have investigated the impact of elevated CO_2 on the control mechanisms of soil C sequestration (i.e. SOM input through plant production and soil C and N dynamics driven by microbial decomposition of SOM).

Soil C input is primarily governed by photosynthesis, which generally increases under elevated CO_2 (Ainsworth & Long, 2005). However, the increased C assimilation under elevated CO_2 may eventually become downregulated as plants need to maintain a balance between N and other resources controlling photosynthesis (Rogers & Humphries, 2000). Indeed, under limited N supply photosynthetic acclimation is more marked, as the capacity of the sinks in plants is too small to utilize the additional photo-assimilates produced under elevated CO_2 (Rogers & Humphries, 2000). Thus, an increase in photosynthesis and concomitant soil C input under elevated CO_2 can partially be inhibited when soil mineral nutrient availability is not sufficient to support plant growth (Vitousek & Howarth, 1991; Bergh *et al.*, 1999).

Elevated CO_2 can decrease or increase soil nutrient availability, depending on the response of the soil microbial community (Norby & Cotrufo, 1998; Torbert *et al.*, 2000; Norby *et al.*, 2001). Diaz *et al.* (1993) proposed

a negative feedback mechanism, where increased C input to the soil from increased productivity in elevated atmospheric CO_2 caused nutrient accumulation in SOM. On the contrary, Zak *et al.* (1993) found decomposition rate to increase after exposure of litter to elevated CO_2 , suggesting that a positive feedback might occur, which would increase rates of nutrient cycling through the ecosystem. In addition, Oren *et al.* (2001) found that a negative feedback in the nutrient cycles induced by elevated CO_2 can be offset when additional N is supplied to the system.

Clearly, the responses of ecosystems to elevated CO_2 have been divergent and C and N dynamics in terrestrial ecosystems depend on a set of complex interactions between soil and plants. It is not clear what the relative importance is of soil C input and soil C mineralization on soil C sequestration under elevated CO_2 . Even if a positive feedback in the C cycle is induced by elevated CO_2 , it is unclear whether this will cause the system to be a source of C. Namely, a disproportionate input of C, though stimulated plant growth may counterbalance C outputs and cause the system to be a sink for C. Also, as the establishment of equilibrium between SOM input and decomposition can take up to decades or longer, we need long-term experiments under realistic field situations to predict changes in ecosystems under future CO_2 levels.

The introduction of open top chambers (OTC) and free air carbon dioxide enrichment (FACE) techniques allowed for long-term CO_2 fumigation studies under realistic growing conditions (Rogers *et al.*, 1983; Hendrey, 1993). Since approximately 20 years, numerous OTC and FACE experiments have been conducted in a broad range of ecosystems. Plant growth and soil characteristics related to C and N cycling have been studied in many of these experiments, but no clear pattern has emerged that allows us to generalize about the effect of rising CO_2 levels on C and N cycling through the plant–soil system (Zak *et al.*, 2000).

Due to high spatial variability and the large size of the soil C pool compared with soil C input, the sensitivity of individual experiments to detect changes in soil C is low (Hungate *et al.*, 1996; Six *et al.*, 2001). A quantitative integration of results across experiments might help to overcome some of these problems. Meta-analytic methods enable placing confidence limits around effect sizes; therefore, they provide a robust statistical test for overall CO_2 effects across multiple studies (Curtis & Wang, 1998). Moreover, they allow testing for significant differences in the CO_2 response between categories of studies (Hedges & Olkin, 1985).

For this review we have compiled the available data from FACE and OTC experiments on plant biomass and a number of soil characteristics related to soil C and N

cycling. Using meta-analysis, we compared the effect of CO₂ enrichment on these variables across some plant functional types and ecosystem management practices. The objective of the analysis is to elucidate whether elevated CO₂ alters nutrient cycling between plants and soil and if so, what the implications are for ecosystem services such as soil C sequestration.

Materials and methods

Database compilation

Data were extracted from 45 studies on plant growth, 59 studies on SOM dynamics and 13 published studies on biological N₂ fixation in FACE and OTC experiments. Data on soil C, N₂ fixation and root biomass were included in a previous meta-analysis (van Groenigen *et al.*, 2006a,b), which compared outdoor facilities (FACE and OTC) with growth chamber and greenhouse studies. In contrast, our analysis focuses solely on OTC and FACE studies. The response variables included in the meta-analysis are listed in Table 1. Values reported in tables were taken directly from the publication, whereas results presented in graphs were digitized and measured to estimate values for the particular pool or flux. Both above- and belowground biomass data were expressed on a dry weight per area basis. When soil density data were available, soil data reported on an area basis were converted to a weight basis. In all other cases, equal bulk soil density in ambient CO₂ and elevated CO₂ treatments were assumed.

Table 1 List of response variables included in the meta-analysis, and their abbreviations used in figures and tables

Response variable	Parameter abbreviation
Aboveground standing plant biomass	APB
Belowground standing plant biomass	BPB
Soil C content	C
Soil C to N ratio	C:N
Microbial C content	MicC
Microbial respiration, measured in short term (<15 days) incubations	rCO ₂
Soil N content	N
Microbial N content	MicN
N mineralization rates, measured in short term (<30 days) incubations	MinN
Gross N immobilization, measured by ¹⁵ N pool dilution methods	GNI
Gross N mineralization, measured by ¹⁵ N pool dilution methods	GNM
Biological N ₂ fixation	N ₂

To make meaningful comparisons between experiments, a number of restrictions were applied to the data. Because of a limited number of studies reporting NPP, only data on total standing above- and belowground plant biomass were included in the analysis. The sampling depth of the belowground biomass ranged from 0–10 to 0–60 cm. When studies reported belowground biomass data in multiple depths, the sum of all depths was used in order to account for the complete root system. With regard to soil data, soil layers ranging in depth from 0–5 to 0–40 cm were included. When data were reported for several depths, the results that best represented the 0–10 cm soil layer were included. For N₂ fixation, all forms of biological N₂ fixation (i.e. free-living and symbiotic bacteria, symbiotic actinomycetes and cyanobacteria) were included. Our review focuses on mineral soils, therefore, measurements on forest litter layers, marsh and rice paddies were excluded from the soil and plant biomass database. The elevated CO₂ levels of the experiments included in the data base ranged from 430 to 750 ppm. Data were not corrected for the degree of CO₂ enrichment. When more than one elevated CO₂ level was included in the experiment, only the results at the level that is approximately twice ambient CO₂ were included. Results from different N treatments, plant species and communities, soils and irrigation treatments within the same experiment were considered independent measurements. These studies were included separately in the database. For OTC experiments, data from the control chambers rather than the nonchamber control plots were included as the results for ambient CO₂. In case these were available, data for blower controls in FACE experiments were included as the results at ambient CO₂.

All root biomass data were obtained by soil coring. Results on C and N fluxes were all based on incubation data (laboratory and *in situ*). Data for microbial biomass were obtained by the fumigation–extraction method (Vance *et al.*, 1987) or the substrate-induced respiration technique (Anderson & Domsch, 1978). The N₂ fixation data were determined by acetylene reduction, ¹⁵N dilution, or N contents of plant tissue when atmospheric N₂ was the only available N source.

For standing plant biomass and soil C and N contents, only the most recent data of each study were incorporated into the database. For data on microbial biomass and activities and N₂ fixation, time series from the most recent year of measurement were included whenever available.

Experimental conditions were summarized by a number of categorical variables: type of exposure facility, N addition and vegetation type (Table 2). We analyzed the interaction between CO₂ and soil N

Table 2 Categorical variables used to summarize experimental conditions, and the values they could assume in the analysis of between group heterogeneity

Categorical variables	Level 1	Level 2
Facility	OTC	FACE
Low vs. high N treatments within studies	Low N	High N
Vegetation	Herbaceous	Woody

OTC, open top chamber; FACE, free air carbon dioxide enrichment.

availability by comparing studies that had received low N vs. high N treatments within the same experiment. For some of the response variables such experiments were underrepresented, in which case we compared between studies receiving low (0–30 kg ha⁻¹ yr⁻¹) or high (>30 kg ha⁻¹ yr⁻¹) levels of N fertilizer. To make statistically meaningful comparisons within categories using meta-analysis, we decided that we need at least 10 data points from at least five different studies. With regard to the N₂ fixation data, we compared studies receiving no mineral fertilization to studies receiving additional mineral (non-N) fertilization. Vegetation was characterized as either herbaceous or woody. The duration of each experiment (i.e. years of CO₂ fumigation) was also included in the database.

Statistical analyses

The data set was analyzed with meta-analytic techniques described by Curtis & Wang (1998) and Ainsworth *et al.* (2002), using the statistical software MetaWin 2.0 (Rosenberg *et al.*, 2000). The natural log of the response ratio ($r = \text{response to elevated CO}_2 / \text{response to ambient CO}_2$) was used as a metric for above- and belowground biomass, C:N ratio's, microbial biomass and activity, soil N mineralization and immobilization rates, and N₂ fixation. It is reported as the percentage change under elevated CO₂ ($[r-1] \times 100$).

In the short term (e.g. decadal), increases in soil C following a rise in soil C input are approximately linear over time (Schlesinger, 1990). As the average duration of CO₂ exposure in the meta-analysis was 3.4 years, we assumed linear accumulation of soil C and N and the natural log of the time-adjusted response ratio $r_t = ([r-1]/yr) + 1$ was used as a metric. Soil C and N results are reported as the percentage change per year under elevated CO₂ ($[r_t-1] \times 100$).

In conventional meta-analyses, each individual observation is weighted by the reciprocal of the mixed-model variance (Curtis & Wang, 1998). However, such an analysis requires that the standard deviations of

individual studies are known. For a large proportion of the observations, these data were not available. Thus, studies were weighted by experimental replication, using the function $F_N = (n_a \times n_e) / (n_a + n_e)$ (Hedges & Olkin, 1985; Adams *et al.*, 1997), where n_a and n_e represent the number of replicates under ambient and elevated CO₂, respectively. We weighted observations of soil C and N by experimental duration and replication, using the function $F_C = (n_a \times n_e) / (n_a + n_e) + (yr \times yr) / (yr + yr)$, with n_a and n_e as before, and yr as the length of the study in years. We choose this metric because well-replicated and long-term studies provide more reliable estimates of effects on soil C and N (Hungate *et al.*, 1996).

Bootstrapping techniques were used to calculate confidence intervals on mean effect size estimates for the whole data set and for categories of studies (Adams *et al.*, 1997). The number of iterations used for bootstrapping was 4999. The CO₂ effect on a response variable was considered significant if the 95% confidence interval did not overlap 0. Means of categories were considered significantly different if their 95% confidence intervals did not overlap.

Results

Plant growth

Both above- and belowground standing biomass increased significantly under elevated CO₂ by 21.5% and 28.3%, respectively (Fig. 1a). Aboveground plant growth was stimulated significantly more under elevated CO₂ for woody species (+30.5%) compared with herbaceous species (+12.6%) (Fig. 1a). A comparable response of woody and herbaceous plant production was observed for belowground biomass, however, the difference was not significant (Fig. 1a).

In contrast to root growth, the aboveground response of plant growth to elevated CO₂ was significantly different between FACE and OTC experiments. The aboveground biomass increase under FACE conditions was 16.5%, whereas CO₂ stimulated plant growth by 27.9% under OTC conditions (Fig. 1a; Table 3). However, the CO₂ pressure used in OTC experiments, particularly for woody species, was generally higher than in FACE experiments (Fig. 2). Also, OTC experiments were heavily biased toward woody species, while herbaceous species made up most of the database for FACE experiments (Fig. 3).

Within experiments that included N availability treatments, high N availability significantly increased the response of aboveground plant growth to elevated CO₂. Aboveground biomass increased by 8.4% under elevated CO₂ following low N availability treatments,

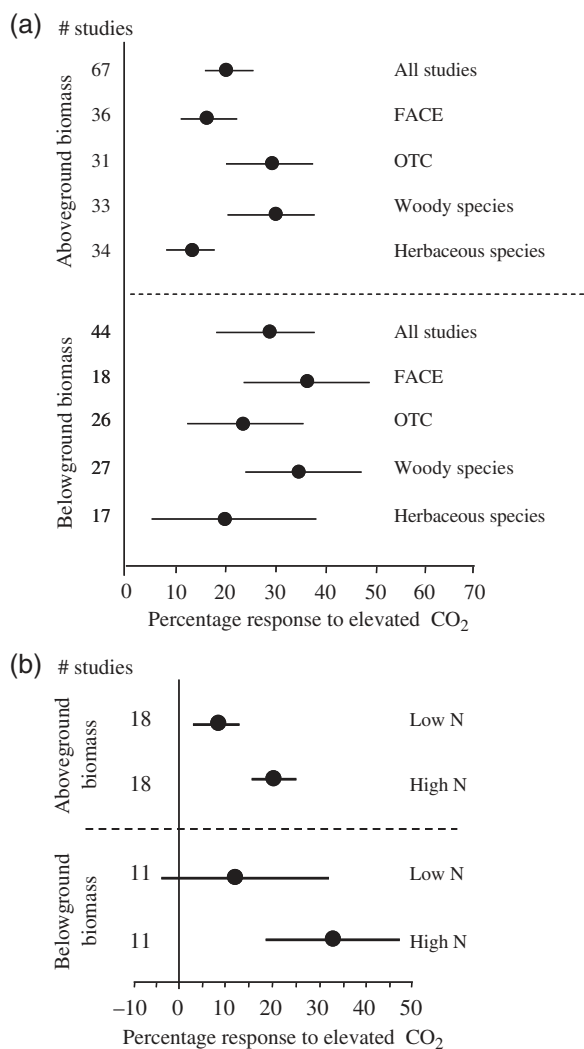


Fig. 1 (a) Percentage response of above- and belowground plant biomass production to elevated CO₂. (b) Percentage response of above- and belowground plant biomass production to elevated CO₂ in low and high N fertilizer treatments.

but the response to elevated CO₂ was significantly higher (+20.1%) under high soil N availability (Fig. 1b; Table 3). The response of root biomass to elevated CO₂ was not significantly different between low and high N availability treatments. However, only in the high N availability treatments did root biomass significantly increase under elevated CO₂ (Fig. 1b). The continuous variable time did not affect the CO₂ response of above- and belowground plant growth in any of the N availability classes (data not shown).

Soil C dynamics

Total soil C increased significantly by 1.2% yr⁻¹ under elevated CO₂, but the CO₂ response depended on soil N

Table 3 Analysis of variance, showing differences in percentage response to elevated CO₂ within the categorized response variables

Response variable	Categorical variable	P-value
APB	Facility	0.032
APB	Vegetation	0.0012
APB	Soil N availability	0.048
BPB	Facility	0.17
BPB	Vegetation	0.18
BPB	Soil N availability	0.19
C	Vegetation	0.71
C	Soil N availability	0.050
C:N	Vegetation	0.081
MicC	Soil N availability	0.25
rCO ₂	Soil N availability	0.15
N	Vegetation	0.038
N	Soil N availability	0.16
MicN	Soil N availability	0.28
N ₂	Soil N availability	0.19

Differences in response are considered significant when P < 0.05.

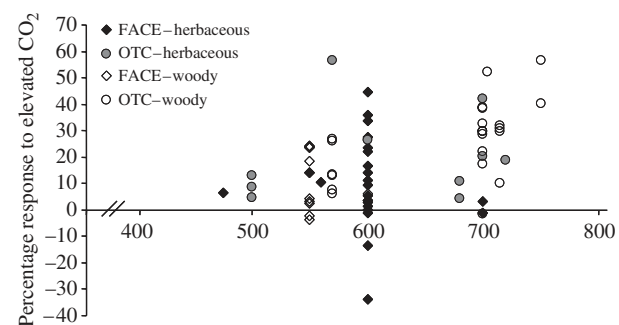


Fig. 2 Percentage response to elevated CO₂ of woody and herbaceous plants as a function of the CO₂ concentrations to which the plants are exposed in free air carbon dioxide enrichment (FACE) and open top chamber (OTC) experiments.

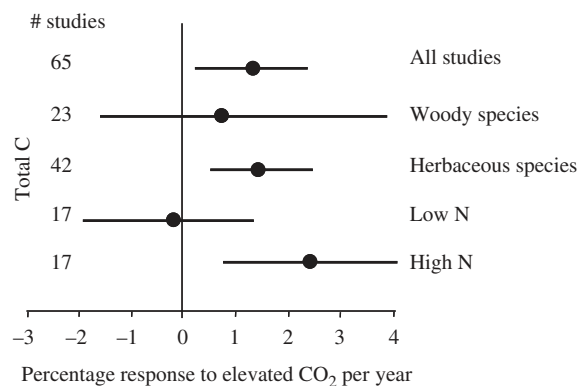


Fig. 3 The yearly response (percentage per year) of soil C contents to elevated CO₂: overall response and categorized soil N availability treatment.

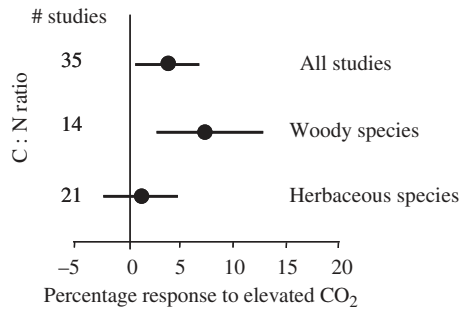


Fig. 4 The percentage response of soil C:N ratios to elevated CO₂: overall response of and categorized by type of vegetation.

availability (Fig. 3; Table 3). Under low N availability soil C contents were significantly lower than under high N availability ($P = 0.05$). In addition, in experiments under low N availability, soil C contents were unaffected, whereas experiments under high soil N availability showed a significant CO₂ response of +2.1% (Fig. 3; Table 3). None of the other categorical variables affected the CO₂ response for soil C contents in the N availability classes. Across the whole soil C data set, the CO₂ response did not differ between herbaceous and woody species. Yet, elevated CO₂ did not significantly affect soil C contents under woody species (+0.89% yr⁻¹), whereas soil C contents under herbaceous species were significantly increased (+1.4% yr⁻¹; Fig. 3).

Averaged over all experiments, the soil C:N ratio increased by 4.1% under elevated CO₂ (Fig. 4). However, only the C:N ratio of the woody species increased significantly under elevated CO₂ (+7.5%, Fig. 4; Table 3). No interactions between CO₂ and N availability treatments were observed for the C:N ratio (data not shown).

Microbial respiration and microbial C increased by 17.1% and 7.7% under elevated CO₂, respectively (Fig. 5). Because of a low number of soil microbial studies conducted under woody species (only five), we were unable to test whether any differences existed in CO₂ response with regard to microbial respiration and microbial C contents, between woody and herbaceous species. The percentage response to elevated CO₂ of either microbial respiration, or microbial C contents, was not affected by N fertilization treatments (Fig. 5; Table 3).

Soil N dynamics

Soil N concentrations did not change under elevated CO₂ (Fig. 6). Under herbaceous species, however, total N increased by 1.0% yr⁻¹ in the high CO₂ environments,

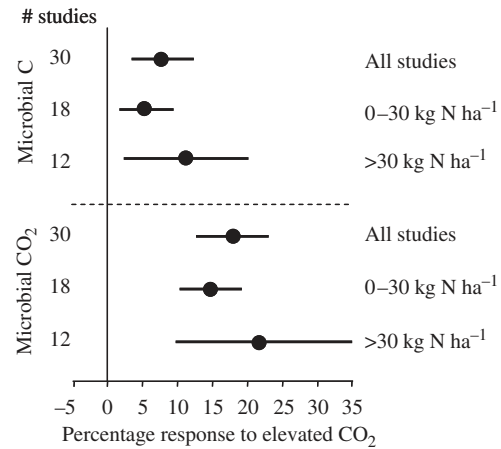


Fig. 5 The percentage response of microbial C contents and microbial respiration to elevated CO₂: Overall response and categorized by low and high N fertilization treatments.

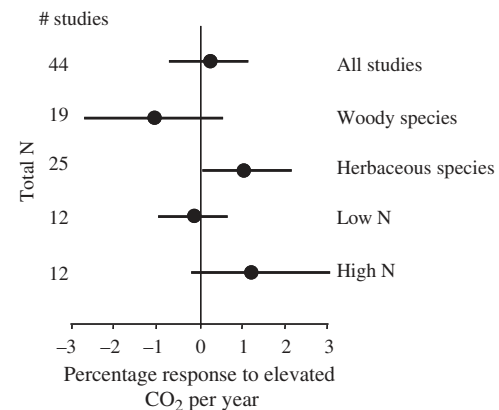


Fig. 6 The yearly response (percentage per year) of soil N contents to elevated CO₂: overall response and categorized by type of vegetation and soil N availability treatment.

which is a significant difference from woody species, which did not respond at all to elevated CO₂ (Fig. 6; Table 3). The response of total soil N to elevated CO₂ was unaffected by N availability treatments (Fig. 6; Table 3).

The effect of elevated CO₂ on microbial N pools and fluxes was characterized by large confidence intervals, indicating large differences among studies in the magnitude of CO₂ responses (Fig. 7a and b). Gross and net N mineralization were not significantly affected by elevated CO₂, whereas gross N immobilization and microbial N contents increased under elevated CO₂ by 22% and by 5.8%, respectively (Fig. 7a and b). No interactions between CO₂ and N availability treatments were found for microbial N contents (Fig. 7b; Table 3). Because of a limited number of studies (ranging from 3

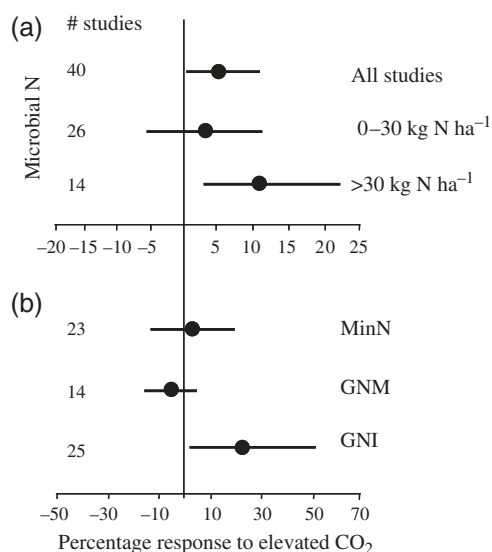


Fig. 7 (a) The percentage response of microbial N contents to elevated CO₂: overall response and categorized by low and high N fertilization treatments. (b) The overall percentage response of gross and net N mineralization and gross immobilization to elevated CO₂.

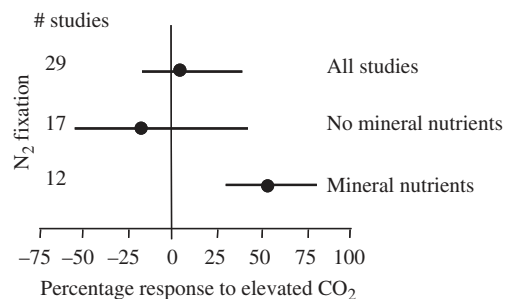


Fig. 8 The percentage response of biological N₂ fixation to elevated CO₂, overall response and categorized by low and high N fertilization treatments.

to 5) conducted on gross and net soil N mineralization and gross N immobilization, we were unable to test whether any differences existed their CO₂ response under different N fertilization treatments (Fig. 7b).

Averaged over all studies biological N₂ fixation did not increase under elevated CO₂. However, N₂ fixation was significantly stimulated (+51%) by elevated CO₂ when additional non-N nutrients were supplied (Fig. 8; Table 3).

Discussion

Plant growth

Overall, elevated CO₂ stimulated aboveground standing biomass by 21.5%, but the increase was significantly

greater for woody than for herbaceous species (Fig. 1a). This corroborates the results of Ainsworth & Long (2005), who found an increase in aboveground plant growth of 20% in a meta-analysis on FACE experiments. Additionally, of all functional groups, they found that trees showed the largest response to elevated CO₂. Several other meta-analyses found no systematic differences between the growth response of herbaceous and woody plants to elevated CO₂ (Curtis & Wang, 1998; Poorter & Perez-Soba, 2001). The discrepancy between results is probably caused by differences in studies included in the meta-analyses, i.e. field vs. pot studies. It has been shown that the biomass increase of herbaceous plants in field studies is considerably smaller than the increase observed in growth chamber studies (Cure & Acock, 1986; Amthor, 2001; Jablonski *et al.*, 2002). Conversely, constraints in growing conditions caused by pots may strongly reduce the growth response to elevated CO₂, particularly for trees (Norby *et al.*, 1999).

The relatively strong growth response to elevated CO₂ of woody compared with herbaceous plants in our analysis may be attributed to the young age of many of the woody species in FACE and OTC experiments (Ainsworth & Long, 2005). Also, most experiments on woody species were conducted in OTC's, which showed a stronger growth response (Fig. 2). However, it should be pointed out that woody species showed a stronger CO₂ response for plant growth than herbaceous species in both FACE and OTC experiments. Thus, the stronger CO₂ response for woody compared with herbaceous species was not an artifact caused by experimental design.

Why did woody species show a stronger response to elevated CO₂ in OTC experiments than in FACE experiments? Firstly, OTC experiments generally apply greater CO₂ concentrations than FACE experiments (Fig. 2). This greater CO₂ concentration is expected to cause a strong growth response, when we assume a linear correlation between plant growth and atmospheric CO₂ concentrations (Kimball *et al.*, 2002). Indeed, we found a significant correlation ($P < 0.01$) between the relatively high CO₂ pressures in OTC experiments and growth responses of woody species to elevated CO₂ (Fig. 2). A second explanation may be that the majority of the data derived from FACE experiments were associated with older trees grown in forest stands, while more studies in OTC experiments were conducted on individual seedlings and young trees. When trees are grown individually, while they are young and there are no constraints on leaf area production, very large CO₂ responses can occur (Norby *et al.*, 1999). Therefore, care should be taken in extrapolating the observed large growth responses of woody species to longer timeframes than current experiment durations

(Norby *et al.*, 1999). To obtain realistic information concerning forest responses to elevated CO₂, we need long-term experiments conducted under natural conditions.

Belowground biomass increased by 28.3% under elevated CO₂ (Fig. 1a). An increase in root biomass is expected to result in higher amounts of C input into the soil through rhizodeposition. Rhizodeposition includes C deposited by living roots in the form of soluble exudates, mucilage, sloughed of root cells or root turnover (Darrah, 1996). An increase in input of root-derived materials under elevated CO₂ likely exerts a more direct effect on soil C and N cycling than aboveground litter input, as root-derived materials are an immediate substrate for microbial activity (Zak *et al.*, 2000). Our results suggest that root biomass is stimulated more than shoot biomass under elevated CO₂. Thus, elevated CO₂ may have greater impact on soil C and N cycling through plant production than predicted from aboveground biomass data alone. However, Nowak *et al.* (2004) directly compared root and shoot growth responses to elevated CO₂ and found that results considering the impact of elevated CO₂ on root:shoot ratios have been inconsistent. Because of a lack of data on root and shoot biomass measured simultaneously within long-term experiments, our analysis can not give a definitive answer with regard to the relative response of roots vs. shoots to elevated CO₂.

Nevertheless, Matamala *et al.* (2003) suggested that not total root biomass, but fine roots are especially important in controlling soil C and N cycling. Norby *et al.* (1999) found that the relative effect of elevated CO₂ on fine root density varied from 60% to 140% in field experiments with trees. Our results indicated an average 40% greater stimulation of fine root biomass under elevated CO₂ compared with total root biomass (data not shown). These results, however, are based on too little observations to allow making statistically meaningful comparisons. Moreover, even when ample data on fine root biomass would be available, differences in fine root longevity and biochemistry between plant species could influence microbial activity in a way that leads to highly variable responses in soil C and N cycling (Zak *et al.*, 2000). Clearly, to gain a more basic understanding of changes in plant–soil interactions under elevated CO₂, an emphasis on root and rhizosphere research in long-term field experiments is needed.

Under elevated CO₂, N availability had a positive effect on both above- and belowground plant growth (Fig. 1b). It has been generally accepted that N additions to systems enhance plant growth responses to elevated CO₂ (Ceulemans & Mousseau, 1994; Kimball *et al.*, 2002; Nowak *et al.*, 2004; Ainsworth & Long, 2005). In fact, it has been proposed that increased plant growth under elevated CO₂ can only be sustained in the long term

when additional N enters the system (Oren *et al.*, 2001, Luo *et al.*, 2004, Reich *et al.*, 2006). Although the growth response of the aboveground biomass to elevated CO₂ was still sustained under low N availability, it declined by 50% compared with systems receiving high N availability treatments. These results suggest that even in relatively short-term experiments low soil N availability strongly limits growth under elevated CO₂, and that the increase in growth under elevated CO₂ in unfertilized systems is expected not to be sustained in the long term.

Soil C dynamics

The overall increase in total soil C under elevated CO₂ suggests a potential for soil C sequestration. However, when taking into consideration the spatial variability in individual field experiments, the average increase of soil C by 1.2% yr⁻¹ is small. Indeed, only a small number of experiments reported a significant impact of elevated CO₂ on soil C sequestration (Rice *et al.*, 1994; Wood *et al.*, 1994; Prior *et al.*, 1997, 2004; Williams *et al.*, 2000; Hagedorn *et al.*, 2001; Six *et al.*, 2001), while some studies showed no differences (van Kessel *et al.*, 2006) and others found decreases in soil C (Calabritto *et al.*, 2002; Cardon *et al.*, 2002; Hoosbeek *et al.*, 2004; Dijkstra *et al.*, 2005).

Several papers have reported a decrease in soil C contents under elevated CO₂. This decrease may have been the result of priming, which is defined as the stimulation of SOM decomposition caused by the addition of labile substrates (Jenkinson *et al.*, 1985; Dalenberg & Jager, 1989). Priming may increase under elevated CO₂ as a result of cometabolic decomposition following a rise in microbial activity (Cheng, 1999). Several studies found that elevated CO₂ affects the decomposition of native SOM (Cardon *et al.*, 2001; Pendall *et al.*, 2003; Hoosbeek *et al.*, 2004). However, as the amount of available native SOM is limited, a CO₂ induced increase in its decomposition is expected to diminish in the long term. Indeed, isotopic data do suggest that CO₂-induced priming is a transient process (M. Hoosbeek, personal communication).

Soil C sequestration significantly increased under elevated CO₂ for herbaceous species only (Fig. 3). However, the larger growth response to elevated CO₂ of woody compared with herbaceous plants implies a greater potential for soil C storage under woody plants. Although, the high variability associated with soil C contents under woody species makes the impact of elevated CO₂ on C sequestration hard to predict (Fig. 4). This variability may be caused by differences in C input, which depends largely on the type (i.e. deciduous vs. evergreen) and the age (i.e. size) of the woody species used and on the type of soil used (Ladegaard-

Pedersen *et al.*, 2005). Alternatively, it has been suggested that trees decrease soil nutrient availability rather quickly under elevated CO₂ (Finzi & Schlesinger, 2002; Barron-Gafford *et al.*, 2005), which may make forests soils prone to priming (Calabritto *et al.*, 2002; Hoosbeek *et al.*, 2004). These factors probably contributed to the relatively strong negative and positive responses of woody species to elevated CO₂, which in combination with the small number of FACE and OTC studies conducted in forests, resulted in high variability.

In our meta-analysis, CO₂ enrichment significantly increased soil microbial C contents and microbial respiration. Microbial activity increased by 17.7% under elevated CO₂ and was not affected by N availability. As soil microorganisms are generally C-limited (Anderson & Domsch, 1978), the increase in C availability, due to increased plant production and concomitant soil C input under elevated CO₂, probably contributed to the greater microbial activity. van Groenigen *et al.* (2006a) showed that the increased input under elevated CO₂ strongly increased labile C pools. As the increase in input is largely counterbalanced by microbial consumption of the shorter lived C pools, the net effect of the additional C input under elevated CO₂ on C sequestration will likely be limited.

van Groenigen *et al.* (2006b) found an increase in soil C only when experiments received additional N fertilization rates of 30 kg ha⁻¹ or more. They suggested that the outcome of their analysis may be confounded by potential differences in initial soil N availability between individual experiments, possibly leading to a misconception of anticipated soil nutrient availabilities based on N fertilization rates. To avoid this confounding factor, we compared only experiments receiving both low and high N fertilization treatments. Our results support the conclusion of van Groenigen *et al.* (2006b) that soil C contents increase under elevated CO₂ only when high N fertilization rates are applied. Apparently, additional N fertilizer stimulates C input (+20.1% and +33.7% for above- and belowground plant growth, respectively) more than microbial activity (+22.3%), leading to greater C accumulation (+2.1% yr⁻¹). Conversely, input under low N availability (+8.8% and +14.6% for above- and belowground plant growth, respectively) is counterbalanced by microbial C respiration (+14.4%). These results support models predicting that N availability may be the limiting factor for C sequestration under prolonged elevated CO₂ (Hungate *et al.*, 2003).

Soil N dynamics

Total N did not increase under elevated CO₂, nor did net N mineralization, gross N mineralization, or N₂

fixation (Figs 6–8). The significant increase in gross N immobilization and microbial N contents under elevated CO₂ suggest a greater N demand by microorganisms. Also, the significant increase in soil C:N ratios under elevated CO₂ suggests a potential decrease in soil N availability. Mikan *et al.* (2000) found that gross N immobilization and plant N uptake by *Populus tremuloides* increased under elevated CO₂, without affecting microbial N contents. Their results suggest that the effect of increased gross N immobilization on soil N availability can be compensated for by enhanced turnover of microbial N. However, we found that microbial N contents increase under elevated CO₂ in long-term experiments, which suggests that over time, an increase in CO₂ will stimulate microbial immobilization of N.

Elevated CO₂ decreased total soil N under woody species, while it increased soil N contents under herbaceous species. In line with these results, the soil C:N ratio increased significantly under woody species only. In several forest ecosystems, soil N leaching decreased under elevated CO₂ (Hungate *et al.*, 1999; Hagedorn *et al.*, 2000; Johnson *et al.*, 2004), suggesting a positive effect of elevated CO₂ on plant N uptake. In corroboration, Barron-Gafford *et al.* (2005) found that increased above- and belowground biomass production of Eastern Cottonwoods in response to 4 years of elevated CO₂, accelerated soil nutrient depletion. These and our data support Finzi & Schlesinger's (2002) prediction that the productivity response of a Sweetgum forest to elevated CO₂ will decline over time as nutrient supplies decrease through increased demands under elevated CO₂.

Overall, N₂ fixation was not affected by elevated levels of atmospheric CO₂. This contradicts results from several short-term experiments investigating the impact of elevated CO₂ on N₂ fixation (Lee *et al.*, 2003). We expected a positive response of legumes to elevated CO₂, as increased rates of photosynthesis increase the C supply to rhizobia (Arnone & Gordon, 1990; Vitousek *et al.*, 2002). In fact, several FACE studies found that legumes, particularly the more effectively nodulating genotypes, showed higher growth responses to elevated CO₂ than other functional groups (Lüscher *et al.*, 2000; Poorter & Navas, 2003; Ainsworth & Long, 2005). Arnone (1999), however, found no effect of elevated CO₂ on N₂ fixation in a late successional alpine sedge community. In agreement with these results, West *et al.* (2005) found no increase in N₂ fixation under elevated CO₂ in three out of four legumes in a multispecies FACE experiment after 4 years of growth. These data suggest that N₂ fixation under elevated CO₂ may, at least for certain leguminous species, not be sustained in the longer term. Niklaus *et al.* (1998) and Hungate *et al.* (2004) suggest that limitation of Mo and P may possibly limit N₂ fixation in long-term FACE experiments. In-

deed, our data show that N_2 fixation increases only under elevated CO_2 when additional non-N nutrients are supplied (van Groenigen *et al.*, 2006b).

Plant–soil interactions under elevated CO_2 , a synthesis

The potential for soil C sequestration to mitigate rising levels of atmospheric CO_2 has stimulated research on C and N cycling in plant–soil systems under elevated CO_2 . Results from studies investigating the impact of elevated CO_2 on soil C and N cycling or on the controlling processes of soil C and N cycling, such as microbial activity, root dynamics and plant growth, have been inconsistent (Norby & Cotrufo, 1998; Zak *et al.*, 2000). These divergent results have generally been attributed to varying environmental and experimental factors. Meta-analysis allows for classifying ecosystem responses to elevated CO_2 and, therefore, to test for the relative importance of these subordinate factors for the potential of soil C sequestration under elevated CO_2 .

Our analysis suggests that the main factor controlling the potential for soil C sequestration under elevated CO_2 is nutrient availability. The pathways by which nutrient availability controls the mechanisms responsible for soil C cycling are depicted in a conceptual diagram (Fig. 9). Overall, our data suggest that on

average, across all systems and fertilization classes, elevated CO_2 stimulated soil C input through increased plant growth (Fig. 9). Even though microbial activity increased by 17.7% under elevated CO_2 , the average increase of 22.5–28.3% in input offset the losses in soil C through increased C mineralization and led to an average 1.2% increase in soil C per year. These results are in agreement with Jastrow *et al.* (2005), who also reported a significant increase in soil C under elevated CO_2 .

However, when classifying ecosystem responses to elevated CO_2 by low and high N availability treatments, we found that soil C sequestration under elevated CO_2 was strongly limited by the availability of nutrients. The lack of C sequestration under low N availability was caused by a reduced response in plant growth and particularly by the reduced stimulation of root growth with elevated CO_2 in low N systems. Namely, the below- and aboveground biomass showed a 8.7–14.6% increase in the low N systems, which was not sufficient to counterbalance the 14.4% increase in microbial activity and resulted in no response of soil C (Fig. 3). In contrast, in the high N systems, the belowground biomass response (+33.7%) exceeded the increase in microbial activity (+20.1%) and the aboveground biomass increase was equivalent to the increase in microbial activity (+22.3). These results show that ample

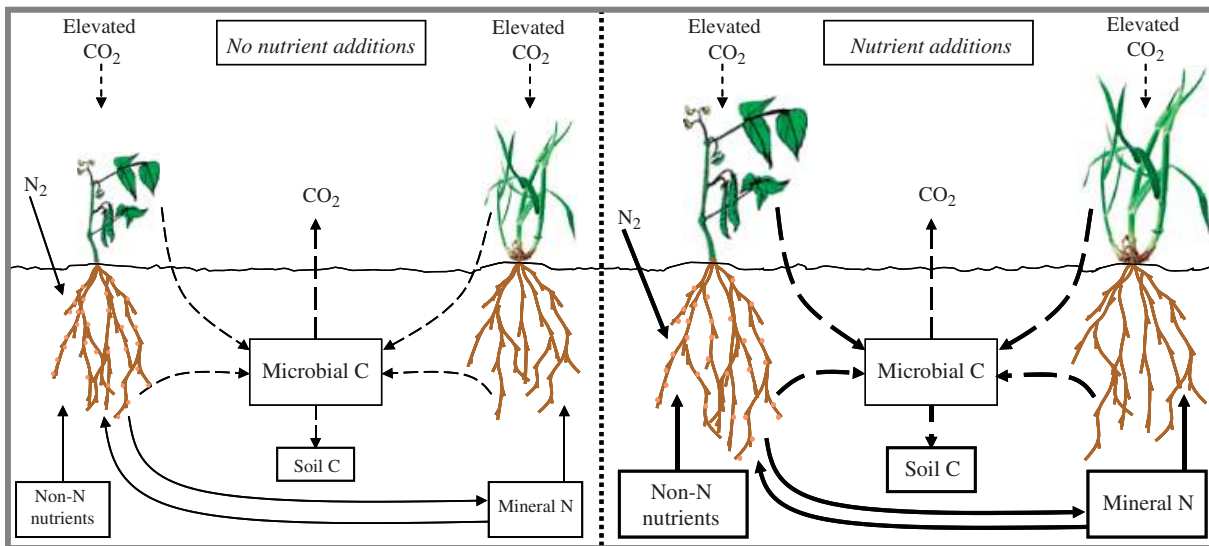


Fig. 9 Conceptual diagram depicting the flows of C and N between plants and soil under elevated atmospheric CO_2 and low vs. high nutrient availability. Soil C sequestration is a function of C input through plant growth and C output through mineralization. Nutrient availability has a limited effect on C mineralization rates under elevated CO_2 . However, the stimulation of above- and below plant growth by elevated CO_2 is larger under high compared with low nutrient availability. Consequently, C input outweighs C mineralization under high nutrient availability, resulting in net C sequestration. On the contrary, when no additional nutrients are supplied the increase in C mineralization under elevated CO_2 counterbalances the increase in C input. Nitrogen fixers can supply additional N needed to sustain plant growth and support C sequestration under elevated CO_2 , but only do so when other nutrients are added. Thus, additional nutrients are required for net C sequestration under elevated CO_2 . Larger boxes represent larger pool sizes and thicker lines represent larger fluxes of C and other nutrients from one pool to the other.

input of root-derived C into the soil is key to sequestering C. As CO₂ respiration increased under elevated CO₂, irrespective of soil N availability, these results suggest that not microbial activity, but rather C input determines the potential for soil C sequestration; C storage is determined by the balance between soil C input and C mineralization, of which the latter showed largely independent and the former is dependent on nutrient availability in ecosystems exposed to elevated CO₂.

In addition to these results, we observed an overall increase in microbial N immobilization under elevated CO₂. These results suggest that in the long term, elevated CO₂ may induce a decrease in soil N availability, which will result in a decrease in plant growth and soil C sequestration. This idea is supported by our and other data on woody plant production (Finzi & Schlesinger, 2002), showing the relatively fast depletion of available N pools under elevated CO₂ caused predominantly by young trees. Overall, this implies that C sequestration in both plants and soils under elevated CO₂ can only be sustained when additional nutrients are supplied (Fig. 9). This conclusion supports the theory of progressive nitrogen limitation (PNL) postulated by Luo *et al.* (2004). In corroboration with this theory a recent study on plant production under long-term elevated CO₂ and different N fertilization rates, also stresses the importance of nutrient additions to grassland systems for sustaining increased plant growth under elevated CO₂ (Reich *et al.*, 2006).

In the short term, PNL can be alleviated or delayed by a number of ecosystem responses. Such responses probably explain the ~10% increase in aboveground plant production under elevated CO₂ and low N availability in our analysis. Firstly, priming has been observed to supply systems with sufficient nutrients to sustain increased plant growth under elevated CO₂ (Hungate *et al.*, 2003). Priming has been shown to occur in nutrient poor soils (Cardon *et al.*, 2001; Pendall *et al.*, 2003; Fontaine *et al.*, 2004), as a mechanism to supply N needed to sustain plant growth under elevated CO₂ (Zak *et al.*, 1993). This mechanism, however, does not contribute to a net gain of ecosystem N, but rather causes redistribution of available N. Redistribution of N under elevated CO₂ is expected to have a limited effect on N availability and soil C sequestration in the long term (Hungate *et al.*, 2003).

Secondly, the efficiency of plant N uptake under elevated CO₂ has been observed to increase due to increased fine root production (Mikan *et al.*, 2000), or increased mycorrhizal colonization of roots (Rillig *et al.*, 2000). However, further increases in plant growth and soil C input resulting from these adaptations, will increase competition for N between plants and mi-

crobes. Thus, CO₂-induced mechanisms that increase plant N uptake without a net ecosystem gain of N are self-limiting (Hungate *et al.*, 2003).

Finally, PNL may be alleviated by the additional supply of N to the soil through N₂ fixation by leguminous plants (Fig. 9). However, our analysis revealed that symbiotic N₂ fixation only increased (50.8%) under longer-term elevated CO₂ when additional non-N nutrients were added. Thus, these results suggest that in unfertilized systems, symbiotic N₂ fixation will have a limited effect on preventing PNL under increased levels of atmospheric CO₂, and that yet again nutrient availability determines the potential for C sequestration under elevated CO₂ (Fig. 9).

In conclusion, we expect that any rapid increases in plant production under elevated CO₂ in unfertilized systems are transient and that the potential for mitigating atmospheric CO₂ through additional soil C sequestration in such systems is minimal (Fig. 9). In contrast, when additional nutrients are supplied we do expect a potential for C sequestration (Fig. 9). The amount of C sequestered, however, seems limited as the increased C inputs are partially counterbalanced by increased C outputs through mineralization. Furthermore, many systems receiving additional nutrients are managed for production of agricultural products. Soil disturbance in these systems may cause a majority of the recently accumulated C to be re-released into the atmosphere (Paustian *et al.*, 2000; Six *et al.*, 2002).

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Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*

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Abstract

Studies on *Fagus sylvatica* show that growth in populations toward the southern limit of this species' distribution is limited strongly by drought. Warming temperatures in the Mediterranean region are expected to exacerbate drought where they are not accompanied by increases in precipitation. We studied levels of annual growth in mature *F. sylvatica* trees over the last half-century in the Montseny Mountains in Catalonia (northeast Spain). Our results show significantly lower growth of mature trees at the lower limit of this species' distribution when compared with trees at higher altitudes. Growth at the lower *Fagus* limit is characterized by a rapid recent decline starting in approximately 1975. By 2003, growth of mature trees had fallen by 49% when compared with predecline levels. This is not an age-related phenomenon, nor is it seen in comparable populations at higher altitudes. Analysis of climate-growth relationships suggests that the observed decline in growth is a result of warming temperatures and that, as precipitation in the region has not increased, precipitation is now insufficient to ameliorate the negative effects of increased temperatures on tree growth. As the climate-response of the studied forest is comparable with that of *F. sylvatica* forests in other southern European regions, it is possible that this growth decline is a more widespread phenomenon. Warming temperatures may lead to a rapid decline in the growth of range-edge populations and a consequent retreat of the species distribution in southern Europe. Assessment of long-term growth trends across the southern range edge of *F. sylvatica* therefore merits further attention.

Keywords: BAI, basal area increment, climate change, drought, European beech, geographical range, Mediterranean, range edge

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Introduction

Changes in growth and reproduction are among the primary responses of trees to environmental variation. In parallel with long-term changes in climate, we expect to see long-term trends in tree growth and reproduction, with consequent impacts on population size and the species' distribution. However, the population-level response of trees to climate is difficult to investigate directly, owing to the long lifespan of individuals and often extended juvenile (nonreproductive) periods (Brubaker, 1986). As individuals of many tree species

may survive for many hundreds of years, assessment of geographical range changes is complicated by their longevity. Adult trees may persist at the edges of the species' distribution long after the climate has become unsuitable for their successful reproduction (LaMarche, 1973; Pigott & Huntley, 1978). Although there are now numerous reports of ongoing climate-related range changes of woody species (see e.g. Wardle & Coleman, 1992; Meshinev *et al.*, 2000; Sturm *et al.*, 2001; Kullman, 2002, 2003; Lloyd & Fastie, 2003; Peñuelas & Boada, 2003; Sanz-Elorza *et al.*, 2003), these are heavily biased toward the leading edge of the species' distribution. As recruitment is generally more sensitive to climate than mortality, an increase in reproduction at the expanding range edge of a tree species' distribution occurs more rapidly than an increase in the mortality of established trees at the retreating edge (LaMarche, 1973;

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Lloyd, 1997). Consequently, it may take decades or even centuries longer to detect range changes at the retreating edge than at the expanding edge, if the same survey-based methods are used to analyse both.

If long-term changes in the growth of established trees are occurring at the range edge, then the trees themselves will record these. Variation in tree growth results in variation in the width of the annual growth rings that are laid down during each growing season. However, ring width in mature trees declines with age, thus, if a declining growth trend is suspected, it may be impossible to investigate it on the basis of changes in ring width alone (Phipps & Whiton, 1988). The conversion of radial increment (ring width) into basal area increment (BAI) overcomes this problem (Phipps & Whiton, 1988; LeBlanc, 1990b; Pedersen, 1998). Unlike ring width, age-related trends in unstandardized BAI are generally positive, culminating in a linear phase of high mature BAI that can be maintained for many decades (Phipps & Whiton, 1988; LeBlanc, 1990b). Therefore, a negative trend in BAI is a strong indication of a true decline in tree growth (LeBlanc, 1990b; Pedersen, 1998).

At the low-altitude and low-latitude range margins of tree species, there is often a negative relationship between growth and growing season temperature and a positive relationship with precipitation, as a consequence of the limiting effects of low water availability on growth and direct effects of extreme temperatures on physiological processes (see e.g. Brubaker, 1986 and references therein; Takahashi *et al.*, 2001 and references therein; Grace *et al.*, 2002; Makinen *et al.*, 2002; Dittmar *et al.*, 2003). The limitation of growth by drought at the low-altitude, low-latitude range edge may have profound consequences for the persistence of populations where current increases in temperature are not matched by increases in precipitation. Investigation of patterns of long-term growth has the potential to identify growth declines long before these become evident as changes in the species distribution.

Recent work by Peñuelas & Boada (2003) has detected an upward shift of *Fagus sylvatica* L. forest in the Montseny Mountains (Catalonia, NE Spain) promoted by the warming climate and changing land use. At its lower altitudinal limit, they report that this species is declining by the gradual isolation and degradation of *F. sylvatica* populations and their replacement by *Quercus ilex* L. (holm oak). Additional work on *F. sylvatica* at Montseny shows a strong negative impact of drought and high summer temperatures on growth (Gutierrez, 1988). The high plasticity of the growth of adult trees enables them to slow growth to survive periods of poor growing conditions; normal growth usually resumes when favourable conditions return (Brubaker, 1986).

Consequently, climatic factors that have a positive or negative effect on annual growth may not be translated into long-term growth trends, unless they are increasing in severity or in frequency over time. Although recent climatic warming has increased the length of the growing season for many species in the Montseny region (Peñuelas *et al.*, 2002), Peñuelas & Boada (2003) report increased discoloration and defoliation of *F. sylvatica* crowns at lower elevations; these are typical symptoms of tree decline (Duchesne *et al.*, 2003).

Given the warming trend reported for the Montseny region and the associated distributional changes of *F. sylvatica*, we aimed to determine whether there is any evidence for changes in recent growth trends of mature individuals. We assessed the effects of climate change on long-term growth of this species by analysing growth trends against yearly climatic variables. Our data provide additional information on whether the decline of isolated populations of *F. sylvatica* at low altitudes involves a general decline of adult trees or whether this decline is more likely to result from regenerative failure in combination with the death of remaining old adults. The localized competitive replacement of *F. sylvatica* by more drought-tolerant species is important in understanding and predicting the fate of this species in the Montseny region. However, significant climate-related growth decline at lower altitudes could have serious implications for the persistence of this species throughout much wider areas of its southern European range-margin.

Materials and methods

Study sites and sample preparation

This work was conducted at the southern edge of the distribution of *F. sylvatica* in Europe in the Montseny Mountains, 50 km NNW of Barcelona (Catalonia, NE Spain; Fig. 1). *F. sylvatica* forest occurs in the temperate zone of the mountains, typically above 1000 metres above sea level (m.a.s.l.). Below the temperate zone the vegetation is Mediterranean, dominated by *Q. ilex* forest. *F. sylvatica* forms the treeline on the highest peaks of the region (Turó de l'Home and Les Agudes, 1712 and 1706 m.a.s.l., respectively). The *F. sylvatica* forest is naturally occurring uneven-aged high forest that has been managed at low intensity by the selective removal of large trees coupled with natural regeneration from seed. However, the impact of forest management on the upper and lower limits of the *F. sylvatica* forest has been low (Peñuelas & Boada, 2003). Soils of the Montseny *F. sylvatica* forest are typically Dystric Regosols and Dystric Cambisols established over schist and granodiorite lithology. The most typical forest community is

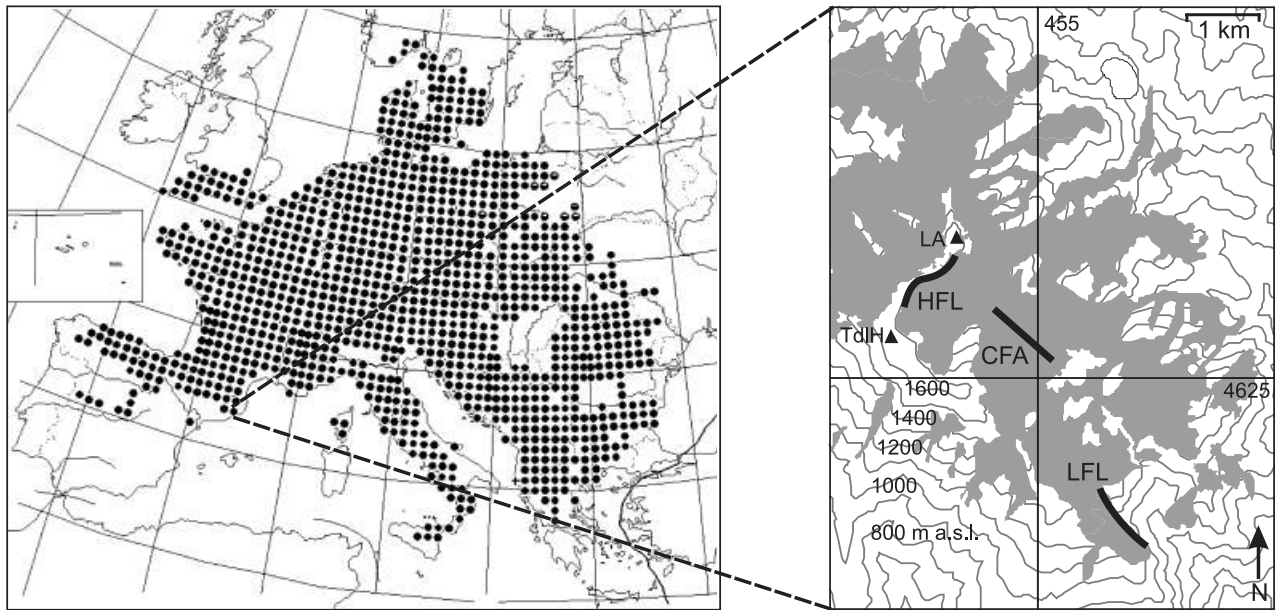


Fig. 1 Left: native distribution of *Fagus sylvatica* (reproduced from Jalas & Suominen (1976), by permission of the Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo). Right: location of study sites in relation to *F. sylvatica* distribution in the study area. *F. sylvatica* distribution is shown in grey. Study sites: HFL, upper treeline (high *Fagus* limit); CFA, central forest area; LFL, low *Fagus* limit. Bold lines show the distribution of individuals sampled at each site. Principal peaks are marked with a triangle. TdlH, Turó de l'Home; LA, Les Agudes. Grid lines are numbered with UTM coordinates in km.

the *Luzulo-Fagetum* with less common areas of *Heleboro-Fagetum*. A detailed description of the vegetation of Montseny and its altitudinal zonation is presented by Bolòs (1983).

Climate data (mean annual temperature and total annual precipitation) were taken from the Turó de l'Home meteorological station (1712 m a.s.l.), directly above our highest sample site (1640 m a.s.l.). Mean annual temperature and total annual precipitation records were available for the period 1952–2003 (Fig. 2). Missing data from the monthly Turó de l'Home climate records for the period 1958–2003 were interpolated from the meteorological stations at Cardedeu (195 m a.s.l., 17 km SSW of Turó de l'Home), Fabra observatory (413 m a.s.l., 48 km SSE) and Roquetes (55 m a.s.l., 194 km WSW) for temperature data and Cardedeu and Fabra observatory for precipitation using MET (Holmes, 2001).

Fifteen mature, dominant or codominant *F. sylvatica* trees without signs of physical damage were selected over a distance of 1 km at each of three altitudinal sites within the largest area of continuous forest. The sites were located in closed forest in areas without signs of recent disturbance and situated at the upper treeline (identified here as the high *Fagus* limit or HFL site), the central forest area (CFA site) and low *Fagus* limit (LFL site; Fig. 1). The HFL and LFL sites were on steep slopes whereas the CFA site was predominantly on level

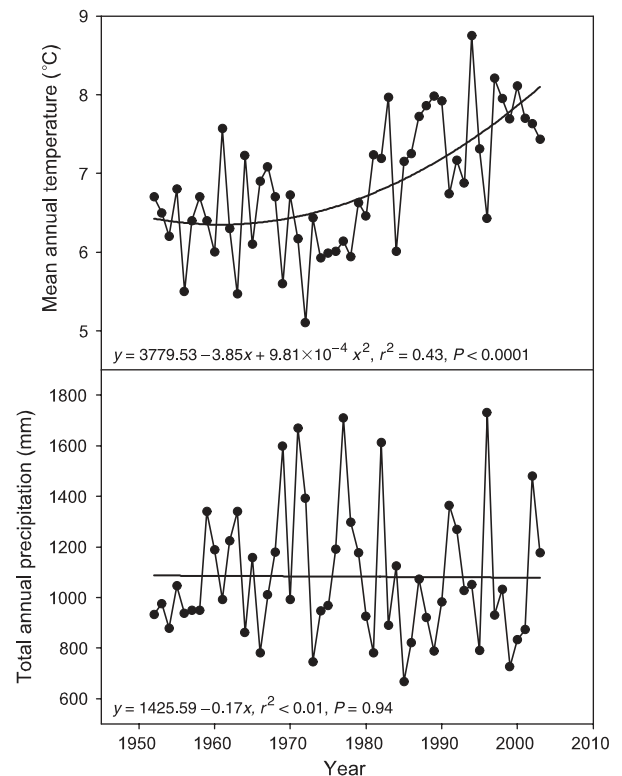


Fig. 2 Mean annual temperature and total annual precipitation at Turó de l'Home during the period 1952–2003. Temperatures show a significant warming trend beginning in the mid-1970s whereas no trend is seen for precipitation.

ground with deeper, less stony soils around the Santa Fe River. All sites were situated on the southeast side of the Turó de l'Home-Les Agudes ridge.

Two or three increment cores were taken from each tree at breast height (1.3 m) using a 4.3 mm increment borer. Diameter at breast height was also recorded. Cores were air-dried and fixed into specially constructed grooved boards. Samples were sanded to prepare them for tree-ring analysis using standard dendroecological methods. Prepared samples were then scanned at 1600 d.p.i. using a flatbed scanner and saved as .jpg files. Ring width was measured to an accuracy of 0.001 mm using CooRecorder v2.3.13 (Larsson, 2003a). Preliminary matching of cores taken from the same tree was performed in CDendro v4.1.1. (Larsson, 2003b). Data files were then converted into decadal format using routines FMT and EDRM of the Dendrochronology Program Library (Holmes, 2001). Cross-dating accuracy was checked using COFECHA v6.06P (Holmes, 2001) and sections of any core that were a poor match with the COFECHA master series for each site were identified. Where poor matching of correctly dated segments resulted from twisted, compressed or decayed wood, these were excluded from the analysis. Ring width for each year was averaged between the cores taken from each tree to produce a final ring width series for each individual. One tree at both the CFA and LFL was excluded from the analysis as these displayed complacent ring-width series (little variation between years) and therefore cross-dated poorly.

Ring width was converted into tree BAI according to the following standard formula:

$$\text{BAI} = \pi(R_n^2 - R_{n-1}^2),$$

where R is the radius of the tree and n is the year of tree ring formation.

To examine the mean growth trend of the dominant and codominant canopy trees for each site, BAI for each year was averaged over all individuals at each site. BAI series for dominant and codominant trees in mature stands typically show a period of early growth suppression (suppression phase) before a rapid increase in annual basal area growth (release phase). BAI may continue to increase in mature healthy mature trees (Phipps & Whiton, 1988; LeBlanc, 1990a,b; Duchesne *et al.*, 2002, 2003; Fekedulegn *et al.*, 2003; Muzika *et al.*, 2004), or stabilize (LeBlanc *et al.*, 1992), but it does not show a decreasing trend until trees begin to senesce (LeBlanc, 1990a; Duchesne *et al.*, 2002, 2003). During the suppression and release phases, growth is strongly affected by variation in light levels and is thus not easily compared between trees. Consequently, we focus only on variation between levels of growth during the

mature phase of growth, marked by the abrupt change in slope after the release phase in our study.

BAI values were also used to estimate age at breast height where none of the cores reached the centre of the tree. After determination of BAI for the full length of the core, a central area of the tree cross-section remained with known area but unknown age. This area was divided by average BAI for the first recorded 15 years of tree growth to give an estimate of the number of years taken to reach this size. This estimated value was added to the number of years of growth recorded for the core to provide an approximate age at breast height.

Tree ring data from each core are usually standardized to remove long-term growth trends and maximize the common climatic signal between trees (Cook *et al.*, 1990). However, for this study it is precisely these long-term growth trends that we were interested in. Consequently, we worked with mean unstandardized BAI values across all trees at each site for each year. These provide interpretable yearly levels of growth rather than a standardized index and display growth trends clearly. They provide the information we seek, but at the expense of the loss of detailed information on climate. Given this low climatic resolution of unstandardized BAI, we analysed the effects of climate on long-term growth using mean annual climate, rather than monthly climatic values as used in dendroecological studies based on ring width. Where we have used a standardization procedure, this was done only to homogenize the variance of data to allow their direct comparison.

Statistical analysis

Mean BAI chronologies for each site were smoothed by the application of a 9-year running average to highlight growth trends while retaining their variability. We used the postrelease inflection point of the 9-year running average to mark the point at which the mature phase begins at each site in our study (Fig. 3). Individual chronologies were visually assessed to ensure that individual trees had reached mature BAI levels by the year indicated by the inflection point of the mean chronology for each site. As the inflection point corresponded to a peak of growth, mature BAI data were analysed from the following year to avoid this peak biasing growth trends away from the null expectation of non-negative mature growth. All statistical analyses use single-year mature BAI data for each site and not smoothed data.

Regression analysis was conducted using SigmaPlot 2002 v8.0 (SPSS Inc., Chicago, IL, USA). All other statistical tests were performed using SPSS for Mac v11.0.2 (SPSS Inc.). The earlier onset of the mature phase

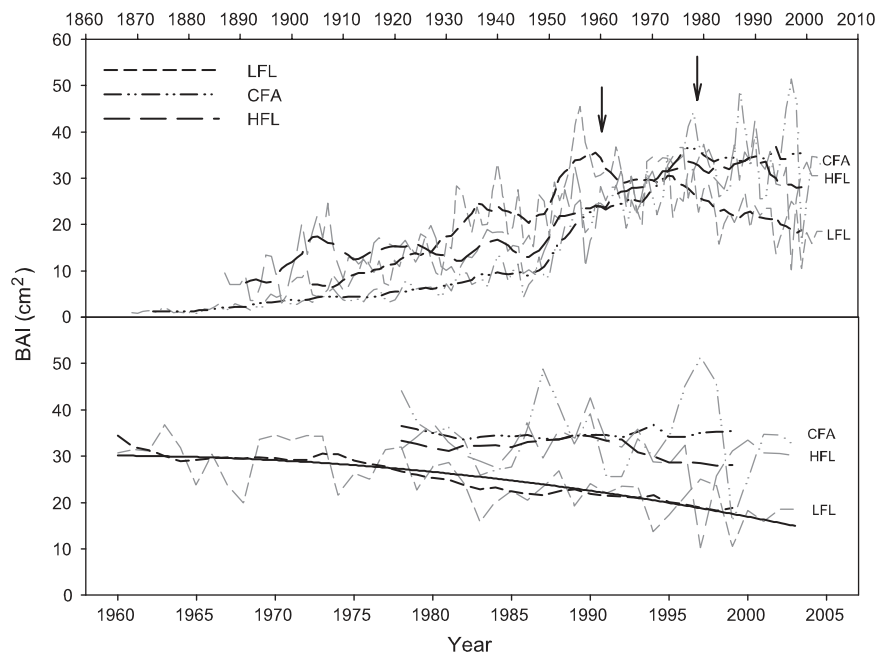


Fig. 3 Mean *Fagus sylvatica* basal area increment for three forest sites differing in altitude. Yearly data are represented by feint lines; trends are highlighted by a 9-year running average in bold. Sites are abbreviated as Fig. 1. Top: full basal area increment (BAI) chronology showing initial suppression phase before increase to mature tree levels. Arrows indicate the beginning of the mature phase of growth at different sites [left: low *Fagus* limit (LFL); right, central forest area and high *Fagus* limit]. Bottom: temporal trends in mature BAI at each site. The significant declining trend of BAI at the LFL site is represented by the solid line ($y = -29565 + 30.22x - 7.71 \times 10^{-3}x^2$, $r^2 = 0.50$, $P < 0.0001$).

in the LFL trees led to different sample sizes (number of years growth) between the three sites (LFL $n = 44$, CFA $n = 26$, HFL $n = 26$). ANOVA was used to test for differences between mean mature BAI at each site. To facilitate comparison, samples at each site were compared for identical periods (1) the 26 years common to all three chronologies and (2) the initial 26 years of the mature phase of growth. This allowed us to control for any unexpected age-related effects leading to differences in growth between sites. When using ANOVA, *a priori t*-test comparisons (not assuming equal variance) were specified to test differences between individual means. ANOVA is relatively robust to unequal variance between samples if sample sizes are constant, however, as there were very large differences in the variance of age and diameter data between samples, the nonparametric (Kruskal–Wallace) test equivalent was used in these comparisons. Linear regression was used to identify significant temporal trends in climate, BAI of individual trees over the 26 years of mature BAI common to all three chronologies and for the average chronology at each site. We assessed first-order autocorrelation (AC) of model residuals in climatic and mature BAI regression analyses using the Durbin–Watson statistic (d) (Durbin & Watson, 1951) and the correlation coefficient r_{AC} calculated according to the formula: $r_{AC} = 1 - (d/2)$.

Inconclusive values of d were treated as indicating significant autocorrelation ($P < 0.05$). Where d indicated significant autocorrelation, we reduced the number of degrees of freedom (df) for each test according to the approximate effective sample size (N_{eff}), calculated according to the formula: $N_{eff} \approx N(1 - r_{AC}) / (1 + r_{AC})$, where N is the sample size (Quenouille, 1952). Treating inconclusive values of d as indicating significant autocorrelation could introduce a Type II error into our results. However, in no case where d was inconclusive did the adjusted df alter the test conclusion in comparison with the nonadjusted df.

For correlation analyses of the relationship between BAI and climate, current year mean annual temperature and total annual precipitation were calculated for period beginning October of the previous year and ending with September of the current year. Previous year climate was summarized for the 12 months immediately preceding this period. This procedure avoids relating growth to climate in the months after leaf fall when growth has ceased. Pearson's product–moment correlation (r) was calculated to identify relationships between BAI and yearly climatic variables and to test for relationships between tree age and diameter and BAI trend and between tree diameter and individual tree mean mature BAI.

To allow direct visual comparison of temporal trends in BAI and related climatic variables, temperature, precipitation and BAI data were standardized to give a standard deviation of 1 and a mean of 0 by subtracting the sample mean from each value of x and then dividing it by the sample standard deviation.

Results

Altitudinal differences in the growth of F. sylvatica

Figure 3 shows that the suppression phase appears to have been less severe at the upper treeline than in the central forest area. At the LFL site, the release phase began much earlier than at higher altitudes, therefore coverage of the suppression phase at the LFL is poor. Likewise, the mature phase began earlier at the LFL than at higher altitudes. There was no significant difference in the mean age of trees at each site (Kruskal–Wallace test, $\chi^2 = 0.01$, $df = 2$, $P = 0.99$) or between tree diameter (Kruskal–Wallace test, $\chi^2 = 0.96$, $df = 2$, $P = 0.62$).

Figure 3 shows that whereas mean annual mature BAI was very similar at the HFL and CFA, the trees of the LFL showed a very different pattern. Starting in approximately 1975, growth dropped rapidly at the lower *Fagus* limit from a mean value of 29.4 cm² for the period 1960–1975 to 14.9 cm² in 2003 – a fall in growth of 49% over a period of 28 years (as indicated by the regression line in Fig. 3). This is evidenced by the significant quadratic relationship between BAI and growth year in the LFL trees ($r^2 = 0.50$, $P < 0.0001$). No significant relationship between mature BAI and growth year was seen at either of the other sample altitudes (CFA: $P = 0.71$; HFL: $P = 0.17$), therefore there is no evidence for any increasing or decreasing growth trend of trees at higher altitudes after they reached maturity in approximately 1978. The significant negative relationship between year of growth and BAI at the LFL persisted if the regression included only the first 26 years of the mature phase in order to compare identical sample sizes between each altitudinal group, ($r^2 = 0.25$, $P = 0.009$, not shown). Tests of the Durbin–Watson statistic (d) revealed no significant first-order autocorrelation in temporal trends of climate or mean mature BAI at the LFL or HFL ($P > 0.05$). Only mean mature BAI at the CFA showed significant autocorrelation among the model residuals ($d = 1.23$, $P < 0.05$, $r_{AC} = 0.385$). When assessing mature BAI trend in individual trees, d was significantly different from 0 in 40% and inconclusive in 19% of individual tests.

Within each stand, the variability in BAI trend among trees was high, as evidenced by the minimum and maximum slope values for the 1978–2003 period shown

in Table 2. At the individual tree level, the majority of trees at both the HFL and CFA showed stable (no trend) or positive trends in mature tree BAI. This situation was reversed at the LFL where the majority of trees showed declining BAI and none showed a positive trend (Table 2). Mature BAI slope of individual trees was not related to tree diameter ($r = -0.10$, $P = 0.55$), but showed a marginally significant negative correlation with estimated tree age ($r = -0.29$, $P = 0.06$).

Average yearly growth over the 1978–2003 period was significantly different between the three forest altitudes (ANOVA: $F_{2,61} = 29.58$, $P < 0.001$) but this difference lay only in paired contrasts involving the LFL. There was no significant difference between mean BAI at the HFL and CFA during this period. When comparisons were made between sites for the first 26 years of the mature phase (comparing the LFL during the years 1960–1985 with the HFL and CFA during the years 1978–2003) the same pattern was evident (ANOVA: $F_{2,61} = 6.41$, $P = 0.003$; Table 2). When calculated over all individual trees, mature tree BAI averaged over the period 1978–2003 showed a significant relationship with tree diameter ($r = 0.56$, $P < 0.001$).

BAI–climate relationships

When comparable sample sizes were considered for all three mature BAI chronologies (for the period 1978–2003), LFL BAI showed a significant negative correlation with temperature in the current and previous year of growth. Significant correlations between BAI and yearly climate were absent at the CFA and HFL (Table 3). When the full 44 years of the mature LFL BAI chronology were analysed, LFL BAI was also correlated positively with both current and previous year precipitation, although the relationship between LFL BAI and precipitation remained weaker than between LFL BAI and temperature.

Unstandardized mean temperature and precipitation for the 1952–2003 period at Turó de l'Home are presented in Fig. 2. There was a clear warming trend of mean annual temperature over this period, beginning in the mid-1970s ($r^2 = 0.43$, $P < 0.0001$), whereas no such trend existed for precipitation ($r^2 < 0.01$, $P = 0.94$). The relationship between LFL mature BAI and current year temperature is shown in Fig. 4a. The relationship with previous year mean temperatures, which exhibit even higher correlations with BAI (Table 3), is identifiable from this figure. In Fig. 4a, current year temperature and LFL BAI track each other very closely over the period analysed. Extreme mean annual temperatures often coincide with and overlap values of high or low growth. It is apparent that the main decline in LFL BAI began in approximately 1975, in parallel with increasing

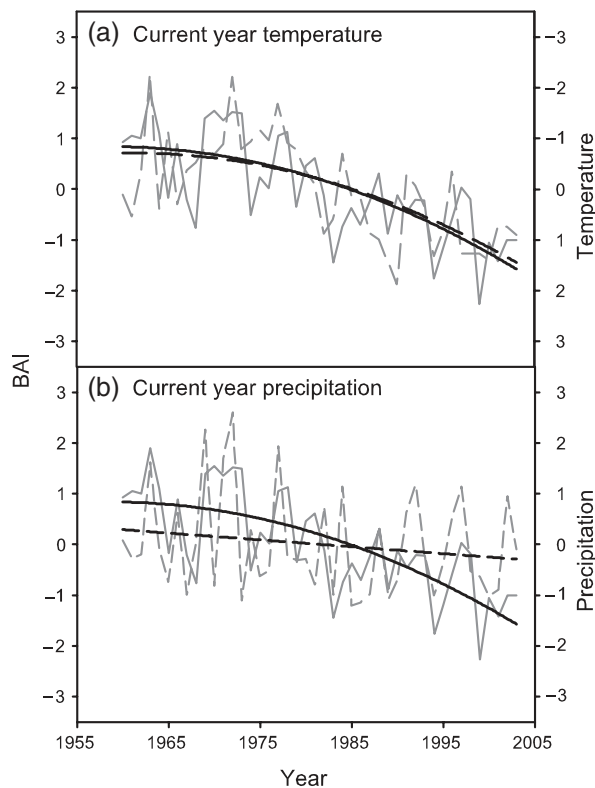


Fig. 4 Temporal trends in standardized current-year climate variables (dashed lines) and standardized mean mature tree basal area increment (BAI) (solid lines) at the low *Fagus* limit. (a) Mean annual temperature; (b) total annual precipitation. (a) The parallel trend in BAI and temperature is highlighted by the regression lines (BAI: $y = -4697.01 + 4.80x - 1.22 \times 10^{-3}x^2$, $r^2 = 0.50$, $P < 0.0001$; temperature: $y = 4702.95 - 4.80x + 1.22 \times 10^{-3}x^2$, $r^2 = 0.43$, $P < 0.0001$). b, Precipitation shows no temporal trend with BAI (BAI: regression as in (a); precipitation: $y = 26.85 - 0.014x$, $r^2 = 0.03$, $P = 0.26$). Annual climate is based on a 12-month period beginning with October of the previous year. Note: The temperature axis is inverted to facilitate comparison with BAI, peaks on the temperature plot therefore represent low temperatures.

mean annual temperature. The standardized chronology for LFL mature BAI and standardized current year total precipitation is presented in Fig. 4b. Unlike the temperature and LFL BAI chronologies in Fig. 4a, the precipitation and LFL BAI chronologies do not track each other consistently well. Agreement between them is good initially, but as BAI is declining whereas precipitation shows no temporal trend, they diverge in recent years. Consequently, although peaks of precipitation often coincide with peaks of BAI or precede them by a year, recent peaks of standardized BAI are usually far lower than peaks of standardized precipitation. Lag effects of previous season conditions are evident in Fig. 4 where extreme climate years precede low or high

BAI by 1 year (e.g. temperature: 1964–1965, 1982–1983, 1996–1997; precipitation: 1967–1968, 1969–1970, 1977–1978) illustrating that both current year and previous year temperatures have a strong impact on levels of growth in the current year.

Discussion

Temporal trends in BAI

Trees at the HFL and CFA show a similar pattern of BAI to that reported for *Liriodendron tulipifera* (yellow poplar), *Quercus prinus* (chestnut oak), *Q. rubra* (northern red oak) and *Acer rubrum* (red maple) by Fekedulegn *et al.* (2003) and for the closely related measure of annual volume increment (AVI) in *Picea rubens* (red spruce) by LeBlanc (1990a,b) and LeBlanc *et al.* (1992) (Fig. 3). Following low BAI during the suppression phase, BAI shows a rapid increase during release before the slope of the curve is reduced as mature tree levels of growth are attained. This reduction in the gradient of the slope marks the beginning of the mature phase in our study (Fig. 3) and is a likely consequence of competition between mature canopy trees (LeBlanc 1990a,b). In our study, *F. sylvatica* maintains steady mean mature BAI at the HFL and CFA, comparable with mature tree AVI in *P. rubens* in the study reported by LeBlanc *et al.* (1992). However, in many species BAI continues to increase with age in mature trees (Phipps & Whitton, 1988; LeBlanc, 1990a,b; Duchesne *et al.*, 2002, 2003; Fekedulegn *et al.*, 2003; Muzika *et al.*, 2004) in some cases showing no evidence of the reduction in slope that LeBlanc (1990a,b) associates with canopy closure.

In contrast with the trees at the CFA and HFL, the LFL trees show a consistent decline of mature tree BAI over the last 28 years of our study. Declining BAI (negative slope) is not an expected consequence of tree maturation (LeBlanc *et al.*, 1992). At least up to 200 years after release from suppression in *P. rubens* (LeBlanc *et al.*, 1992) and up to 350 years in *Quercus alba* (white oak; Phipps & Whitton, 1988) there is no evidence of negative BAI trends in healthy trees. Therefore, negative BAI trend is a strong indicator of a true decline in tree growth (Phipps & Whitton, 1988; LeBlanc, 1990a,b; Duchesne *et al.*, 2002, 2003). Prolonged BAI decline is naturally associated with tree senescence (LeBlanc, 1990a; Duchesne *et al.*, 2002, 2003) and a good indicator of impending death (Pedersen, 1998). BAI may decline in response to a variety of stresses including competition (Duchesne *et al.*, 2002), insect outbreaks and drought (Pedersen, 1998; Hogg *et al.*, 2002), atmospheric pollution (Duchesne *et al.*, 2002; Muzika *et al.*, 2004)

or other unidentified atmospheric stresses (Phipps & Whiton, 1988; LeBlanc, 1990a; LeBlanc *et al.*, 1992).

BAI decline and temperature

In our study, mature BAI trend of individual trees shows a marginally significant declining trend with increasing tree age ($r = -0.29$, $P = 0.06$). As most negative individual BAI trends occur at the LFL this may indicate that older trees are more susceptible to growth decline. Given the similar mean age and history of the three stands we studied and the similar stand structure of the three sites (Table 1), the declining mean mature BAI trend at the LFL cannot be interpreted as a natural phenomenon associated with tree age or stand dynamics. Nor is it an artefact of comparing BAI chronologies of different lengths, as the phenomenon remains if the same analysis period of 26 years is compared across all sites (see 'Results' and Table 2). BAI at the LFL shows a strong negative relationship with mean annual temperature (Table 3) in agreement with the climate response reported for *F. sylvatica* from Turó de l'Home in the dendroecological study of Gutierrez (1988). The close correspondence of the declining BAI trend at the LFL with that of temperature recorded at the Turó

de l'Home meteorological station (Fig. 4) shows that declining growth at the LFL may be closely linked to the rising temperatures of the Montseny region.

F. sylvatica is a masting species and seed production shows strong links with climate (Piovesan & Adams, 2001). Despite the potential for reduced BAI in mast years, the close proximity of our sample sites to one another (Fig. 1) suggests that the decline in BAI at the LFL cannot be adequately explained by increased masting frequency in this region. Growth of *F. sylvatica* at low altitudes at Turó de l'Home is limited by high growing-season temperatures and promoted by high precipitation during the growing season (Gutierrez, 1988; A. S. Jump *et al.*, unpublished). This is consistent with the high sensitivity of this species to drought (Gutierrez, 1988; Dittmar *et al.*, 2003; Lebourgeois *et al.*, 2005) and in part explains the climate-growth responses seen in Fig. 4 and Table 3. Furthermore, in parallel with recent warming, positive effects of high precipitation appear to have declined relative to the negative effects of temperature in recent years (Fig. 4, A. S. Jump *et al.*, unpublished). The susceptibility of *F. sylvatica* to extreme temperatures increases with decreasing soil water availability (Lebourgeois *et al.*, 2005), which is a likely consequence of increasing temperature

Table 1 Summary information for *Fagus sylvatica* samples used in this study

Site	Stand basal area (m ² ha ⁻¹)	Leaf area index	Altitude (m a.s.l.)	N	DBH (cm)	Age (years)
Upper treeline (HFL, high <i>Fagus</i> limit)	23.98	4.06	1640	15	56.6 (28.2–118.4)	110.47 (50–236)
Central forest area (CFA)	25.24	4.30	1127	14	47.3 (25.8–66.2)	94.6 (57–143)
Low <i>Fagus</i> limit (LFL)	20.62	4.09	992	14	51.3 (33.3–68.1)	92.1 (50–119)

Mean values for DBH and age are followed by the range in parentheses. DBH and age do not differ significantly between sites (Kruskal–Wallace test, $P > 0.05$).

Altitude, mean sample altitude; N, number of trees analysed; DBH, diameter at breast height; Age, age at breast height.

Table 2 Comparison of mature tree basal area increment (BAI) across common 26-year intervals

Site	Linear BAI trend 1978–2003					
	Average chronology		Individual chronologies		Mean mature BAI (cm ²)	
	Slope	P (linear regression)	Range of slopes	Direction of slopes	1978–2003	First 26 years of mature period
HFL	-0.20 (0.18)	0.17	-2.04–1.14	2 [+], 10 [NT], 3 [-]	31.36 ^a (1.09)	31.36 ^a (1.09)
CFA	-0.10 (0.12)	0.71	-1.24–0.34	1 [+], 10 [NT], 3 [-]	34.53 ^a (1.61)	34.53 ^a (1.61)
LFL	-0.36 (0.12)	<0.001	-1.58–-0.26	6 [NT], 8 [-]	21.50 ^b (0.94)	27.99 ^b (1.09)

Sites abbreviated as in Table 1. Standard error is given in parentheses.

Direction of slopes: [+]/[-], significant positive/negative trend at $P \leq 0.05$; [NT], no significant trend ($P > 0.05$).

Mean mature BAI in each column followed by different letters differs significantly (*t*-test comparisons, $P < 0.05$).

For explanation of 26-year periods see text.

Table 3 Correlation of *Fagus sylvatica* BAI with mean annual temperature and total annual precipitation in the current and previous year

Site	Period	Previous year		Current year	
		Temperature	Precipitation	Temperature	Precipitation
HFL	1978–2003	–0.09 (0.66)	–0.21 (0.32)	0.02 (0.92)	–0.13 (0.53)
CFA	1978–2003	–0.27 (0.40)	–0.08 (0.80)	–0.01 (0.97)	0.11 (0.73)
LFL	1978–2003	–0.61 (0.001)	0.27 (0.18)	–0.45 (0.022)	0.25 (0.22)
LFL	1960–2003	–0.62 (<0.001)	0.42 (0.004)	–0.59 (<0.001)	0.43 (0.004)

Sites abbreviated as in Table 1.

Climate-BAI correlations for the LFL site are given for the 1978–2003 interval common to all sites and for the full mature BAI chronology.

P values are given in parentheses, significant correlations are presented in bold type.

HFL, high *Fagus* limit; CFA, central forest area; LFL, low *Fagus* limit; BAI, basal area increment.

but unaltered precipitation recorded for the Montseny region (Fig. 2). A reduction in days when fog is recorded at the Turó de l'Home meteorological station has accompanied warming temperatures (data not shown), which suggests that fogs should also be shifting to progressively higher altitudes and occurring less frequently at the LFL. Fog episodes may have a positive effect on the growth of *F. sylvatica* in Montseny (Bolòs, 1983; Gutierrez, 1988), although they are not essential for this species to persist (Peñuelas & Boada, 2003). Unfortunately, we have no data on the occurrence and duration of fog episodes at the lower limits of the *F. sylvatica* forest.

BAI related to altitude

Trees in the central forest area show a very similar response to climate to those of trees at lower altitudes (Gutierrez, 1988; A. S. Jump *et al.*, unpublished). They show a comparably strong negative response to growing season temperatures, although precipitation at the CFA is not as limiting as at the LFL. Our CFA site is, on average, 135 m higher in altitude than the LFL – corresponding to an approximate temperature decrease of 0.69 °C (from the altitudinal temperature lapse rate of 0.51 °C per 100 m reported by Peñuelas & Boada (2003) for Montseny). Given the increase in mean annual temperatures at Montseny shown in Fig. 2, mean annual temperatures at the LFL in 1975 should equal those experienced at the CFA approximately 12 years later. If calculated only on the basis of difference in altitude, this indicates that the growth decline that began at the LFL some 28 years ago should have been affecting this central area of *F. sylvatica* forest for approximately the last 16 years. The CFA site is located in a level area of forest around the Santa Fe River and has deeper soils than those of the steep northeast-facing slope of the LFL

(see Bolòs, 1983). Lebourgeois *et al.* (2005) show that soil water deficits play a crucial role in limiting the growth of *F. sylvatica* and moderating the effects of high temperatures. Differences in soil water availability between the two sites may explain why growth of the CFA remains unchanged while it is declining rapidly at the LFL – an explanation supported by the lower importance of high precipitation in promoting growth in the central forest area. Based on temperature differences between sites, our data suggest any future increase in aridity could cause growth to decline over much larger areas of the *F. sylvatica* forest at Montseny. Further study of differences in soil hydrology between these two sites would be rewarding.

Paulsen *et al.* (2000) note that a reduction in radial growth approaching the upper treeline can be interpreted as a direct response to decreasing temperature with increasing elevation. In their study of *Pinus cembra* and *Picea abies* they found evidence for an historic altitude-dependent reduction in annual radial growth over a distance of 250 m altitude approaching the treeline in the European Alps. The expected reduction in growth toward the treeline suggests that mature tree BAI should be lower at the HFL than at the CFA some 500 m below. However, Paulsen *et al.* (2000) describe a major increase in the radial growth of trees at the alpine treeline since 1940 related to increased temperature, with current growth at the treeline now equal to that of trees at sites 250 m lower.

If temperature increases are responsible for decreasing BAI at the LFL in our study then we might expect recent temperature increases to have increased BAI at the HFL, as high temperatures are linked to high annual growth at this site. The CFA trees are our best indicator for the maximum BAI achievable for these forest sites as this site occurs on deeper soils away from the climatic extremes experienced at the forest's altitudinal margins.

Current levels of mean BAI are similar for the CFA and HFL samples (Table 2) as are mean tree age and diameter at breast height (Table 1). As growth at the upper treeline is expected to be lower than at lower elevations (Paulsen *et al.*, 2000), a recent increase in BAI at the HFL may have taken place. Unfortunately, as we have no evidence for historic mature tree levels of BAI at the HFL, our data do not currently allow us to assess whether any recent increase in growth at this site has occurred.

Wider significance of F. sylvatica growth decline

When considered alongside the high sensitivity of *F. sylvatica* to drought (Dittmar *et al.*, 2003; Lebourgeois *et al.*, 2005) and the recent increase in its sensitivity to temperature (A. S. Jump *et al.*, unpublished), the data we present here demonstrate a dramatic growth decline at the LFL related to rising temperatures in the study region over the last half-century. Strikingly, in a study of old-growth *F. sylvatica* forest in the Central Apennines, Italy, Piovesan *et al.* (2005b) report a comparable growth decline of this species over the same period, which they suggest may be related to changes in climate. In their study, BAI drops from approximately 28 cm² in 1975 to approximately 21 cm² in 2000 – a fall of 25% over 25 years. Although Piovesan *et al.* (2005b) do not specifically investigate the relationship between climate and BAI in the studied population, previous work found positive effects of spring temperature and summer precipitation on *F. sylvatica* ring width in the same area over the period 1832–2000 (Piovesan *et al.*, 2003). Throughout the Central Italian Apennines, low-frequency variation in ring width has been related to variation in the North Atlantic oscillation (Piovesan & Schirone, 2000). Like the *F. sylvatica* forest we investigated, the forest studied by Piovesan *et al.* (2005b) is also from the Mediterranean region and close to the southern range-edge of this species' distribution. Growth of *F. sylvatica* is strongly drought limited across the southern region of its distribution (Gutierrez, 1988; Biondi, 1993; Biondi & Visani, 1996; Dittmar *et al.*, 2003; Piovesan *et al.*, 2003, 2005a; Lebourgeois *et al.*, 2005). The comparable fall in BAI reported by Piovesan *et al.* (2005b) suggests that this climate-related decline in the growth of *F. sylvatica* at the edge of its range in Montseny is not an isolated phenomenon, but may be occurring at the southern range edge of this species elsewhere in Europe.

F. sylvatica is an increasingly important species for European forestry (Dittmar *et al.*, 2003) and therefore a highly significant species in terms of carbon sequestration (Bascietto *et al.*, 2004). Recent climatic warming has increased the length of the growing season for many species in the Montseny region (Peñuelas *et al.*, 2002),

suggesting a positive growth response to recent climatic changes might be expected for *F. sylvatica*. Many studies in recent years describe increasing growth trends for *F. sylvatica* and other forest species throughout Europe, leading to the assumption that carbon sequestration by this species is increasing (Sabaté *et al.*, 2002; Dittmar *et al.*, 2003; Bascietto *et al.*, 2004 and references therein). Our results demonstrate that for some low-altitude and low-latitude populations the reverse may be true, with increasing temperatures resulting in recent and rapid decline of the growth of this species.

Conclusions

Previous work on *F. sylvatica* in the Montseny Mountains by Peñuelas & Boada (2003) found evidence that this species may be in decline at low altitude range edge. The work that we present here shows that this decline is linked to a rapid and recent decrease in the growth of adult trees at the lower limit of this species' distribution. Growth at the lower limit of *F. sylvatica* in this region fell by 49% between 1975 and 2003 linked to recent climatic warming. Our evidence suggests that increasing temperatures are exacerbating drought, with the result that the positive effects of years with high annual precipitation are declining. We cannot predict the long-term growth response of the forest interior based on temperature alone, but our results indicate that only a small increase in aridity may decrease growth of this species over much larger areas. Determining whether more widespread growth decline of *F. sylvatica* is occurring at its southern range edge should be the focus of further research.

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Earth by comets and meteorites. Further studies of these objects may elucidate whether their composition and membrane-like structures were important building blocks for the origin of life.

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Supporting Online Material

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Materials and Methods
Fig. S1
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Increasing Trend of Extreme Rain Events Over India in a Warming Environment

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Against a backdrop of rising global surface temperature, the stability of the Indian monsoon rainfall over the past century has been a puzzle. By using a daily rainfall data set, we show (i) significant rising trends in the frequency and the magnitude of extreme rain events and (ii) a significant decreasing trend in the frequency of moderate events over central India during the monsoon seasons from 1951 to 2000. The seasonal mean rainfall does not show a significant trend, because the contribution from increasing heavy events is offset by decreasing moderate events. A substantial increase in hazards related to heavy rain is expected over central India in the future.

Analysis of rain gauge data shows that Indian monsoon rainfall has remained stable over the past century even though the global mean surface temperature has risen steadily (1–3). Although the amount of summer monsoon rain [June to September (JJAS) seasonal mean all-India rainfall, AIR] has some interdecadal variability (4), it has no significant long-term trend (Fig. 1). Physical considerations and model studies indicate that tropospheric warming leads to an enhancement of moisture content of the atmosphere (5) and is associated with an increase in heavy rainfall events (6–11).

Extreme rainfall results in landslides, flash floods, and crop damage that have major impacts on society, the economy, and the environment. Although prediction of such extreme weather events is still fraught with uncertainties, a proper assessment of likely future trends would help in setting up infrastructure for disaster preparedness.

The number of severe cyclonic storms over the north Indian Ocean (IO) has shown an increasing trend in the past 3 decades (12, 13), consistent with similar findings over other basins (12). However, no coherent signal has emerged from investigations of the trend of daily station rainfall data over India (13–16), with some stations showing an increasing trend whereas others show a decreasing trend. The ambiguity in the existence of a trend in monsoon rainfall extremes may be partly related to

the data and the methodologies used so far. Short-duration extreme rain events are a consequence of small-scale convective instabilities in a moist atmosphere. Although a fraction of extreme rain events is triggered in the background of synoptic disturbances (17) and is preferentially located around the tracks of monsoon lows and depressions, a large fraction arises from processes like severe thunderstorms and is more uniformly distributed in space and time. Even if the total number of extreme events over a homogeneous large-scale environment were to have an increasing trend, no significant trend may appear in data from a single station because of the inherently large variability and/or sampling issues (18–23). Therefore, we examined the trend of daily heavy and very heavy rain events over a relatively large region.

We used daily gridded rainfall data at 1°-by-1° resolution from the India Meteorological Department (IMD), based on 1803 stations (24, 25) that have at least 90% data availability, for the period 1951–2000. The interannual variability of JJAS all-India rainfall (AIR2) from this data set (Fig. 1B) is similar to AIR, which is a long-term data set based on 306 stations (26). Daily anomalies of rainfall at each grid box were constructed as deviations of observed daily values from a smoothed climatological annual cycle (the sum of the mean and first three harmonics of the daily climatology). The climatological mean and variance of daily summer monsoon rainfall have large spatial variability across the country (Fig. 1A and fig. S1). However, over central India (CI, 74.5°E to 86.5°E and 16.5°N to 26.5°N, containing 143 grid boxes) the mean and the standard deviation are reasonably homogeneous (spatially

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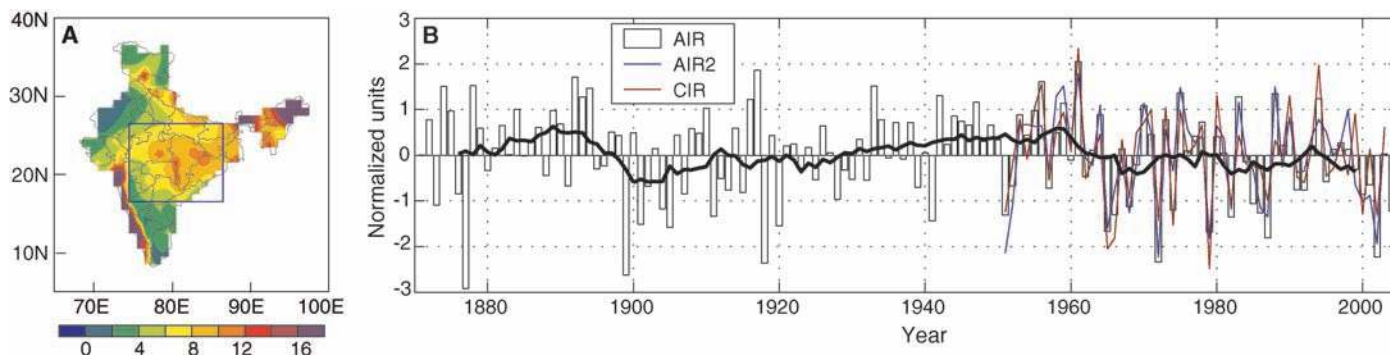


Fig. 1. (A) Climatological mean summer monsoon rainfall (mm/day). The box indicates the CI region used in our analysis. (B) Normalized (by the interannual standard deviation) JJAS AIR based on 306 stations (26) from 1871 to 2003 (bars). The mean is 84.9 cm, and the standard deviation is 8.4 cm. The solid black line represents an 11-year running mean indicating interdecadal variability but

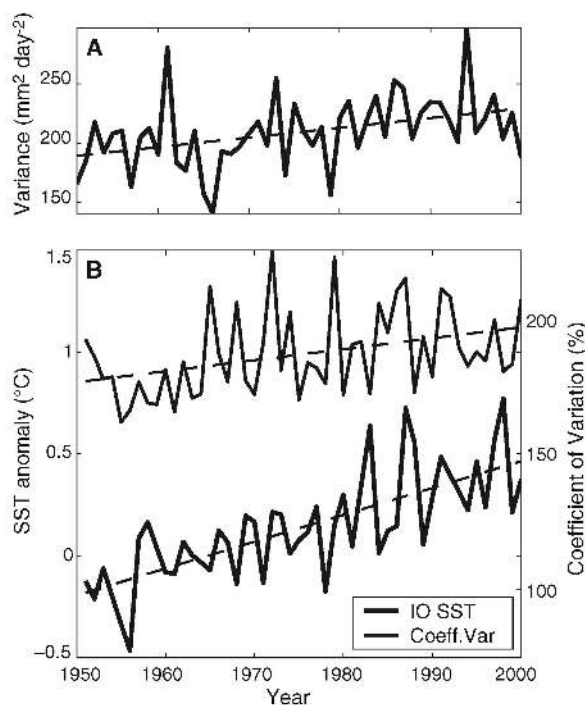
no trend. The AIR2 (blue) is the normalized seasonal mean AIR on the basis of the new gridded rainfall data (24). The seasonal mean and standard deviation are 94.0 cm and 9.1 cm, respectively. The CIR (red) is the normalized seasonal mean over CI on the basis of the gridded rainfall data set, the mean and the standard deviation of which are 69.5 cm and 11.2 cm, respectively.

uniform). Therefore, we select CI as the region to examine the trend of extreme rainfall over India.

The gridded daily data are smoother than the individual station data because of averaging over a 1° -by- 1° box. The maximum 1-day rainfall during the summer monsoons of 1951 to 2003 in any box over CI is 58.2 cm. The seasonal mean over CI is 5.7 mm of rain in a day (mm/day), whereas the standard deviation of the daily anomalies is 11.5 mm/day. Although a fixed threshold for defining extreme events is not appropriate over regions where the mean climate has large spatial variability (27, 28), a fixed threshold can be used to define extreme rain events over CI, where the seasonal mean climate as well as the daily variability is reasonably homogeneous (Fig. 1A and fig. S1). We used 100 mm/day in a 1° -by- 1° box as a threshold to define a heavy rain event, whereas a threshold of 150 mm/day was used to define a very heavy event.

The temporal variance of daily rainfall anomalies averaged over CI shows a significant increasing trend (at 0.01 significance level) during 1951 to 2000 (Fig. 2A). The increasing trend of the coefficient of variability, defined as the ratio of the standard deviation to the mean, of daily monsoon rainfall (Fig. 2B) is a consequence of the absence of a trend in the seasonal mean (Fig. 1) and an increasing trend in the standard deviation. A trend in daily rainfall variance is related to a trend in large-scale moisture availability (5), which in turn is due to gradual warming of sea surface temperature (SST) (7). However, interannual changes in moisture content over CI can be influenced by regional-scale land surface processes as well as by atmospheric teleconnections associated with remote SST such as the El Niño and Southern Oscillation (ENSO). Although El Niño events are generally associated with positive SST anomaly over the tropical IO, they lead to drying of the atmosphere over CI through large-scale subsidence. As a result, daily CI rainfall variance and IO SST need not

Fig. 2. (A) Temporal variation (1951 to 2000) in the variance of daily anomalies during summer monsoon seasons (June 1 to September 30), together with its linear trend (dashed line). (B) Coefficient of variability of daily precipitation during summer monsoon season and its trend (thin line) together with JJAS SST anomalies averaged over tropical IO and their trend (bold line). Statistically significant trends (0.01 significance level) are calculated on the basis of a *t* test, with a sample size of 50, under a null hypothesis of no trend.



be correlated on a year-to-year basis. The long-term increase of daily rainfall variance is likely due to the warming trend of tropical IO JJAS SST (Fig. 2) and the associated increase in water vapor (5).

The frequency histogram of daily rainfall at each 1° by 1° box (R) over CI during the summer monsoons of 1951 to 1970 and 1981 to 2000 was separately constructed (plotted as line curves in fig. S2) to assess the increase in variance in recent decades compared with those of the 1950s and 1960s. The tails of the histogram indicate a larger number of extreme events (≥ 100 mm/day of rain) during 1981–2000. On the other hand, the number of light to moderate events (≥ 5 mm/day but < 100 mm/day) have decreased during 1981 to 2000 compared with 1951 to 1970. In fact, the frequency of heavy

($R \geq 100$ mm/day) and very heavy ($R \geq 150$ mm/day) events over CI shows clear and significant (at 0.01 significance level) increasing trends (Fig. 3) (29), whereas that of moderate events shows a significant (at 0.1 significance level) decreasing trend. There is a 10% increase per decade in the level of heavy rainfall activity since the early 1950s (Fig. 3A), whereas the number of very heavy events has more than doubled (Fig. 3B), indicating a large increase in disaster potential. These findings are in tune with model projections (6–11) and some observations (30) that indicate an increase in heavy rain events and a decrease in weak events under global warming scenarios.

In order to see whether the unambiguous increase in the frequency of heavy and very heavy events is also accompanied by an increase in the

intensity of heavy events, we examined the rain intensity between 99 and 99.99 percentiles (31) of summer monsoon rainfall (Fig. 4A). The rainfall intensity that contributed to the 99.75 percentile in the early 1950s seems to contribute to only the 99.5 percentile in the early 1990s,

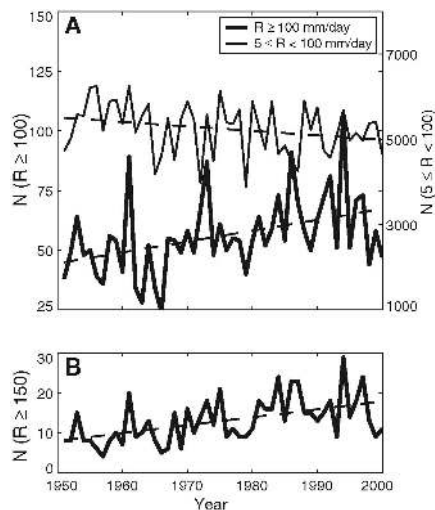


Fig. 3. Temporal variation (1951 to 2000) in the number (N) of (A) heavy ($R \geq 100$ mm/day, bold line) and moderate ($5 \leq R < 100$ mm/day, thin line) daily rain events and (B) very heavy events ($R \geq 150$ mm/day) during the summer monsoon season over CI. The statistical significance of the trends (dashed lines) was calculated as in Fig. 2.

with events of higher intensity contributing to the higher percentiles. For instance, the average intensity of the heaviest four events in each monsoon season (Fig. 4B) shows an ~10% per decade increase over the 50-year period (18 to 26 cm), significant at 0.01 significance level.

Although the above results present strong evidence of an increase in the number of extreme monsoon weather events over India over the past half century, the Indian monsoon climate (seasonal mean monsoon rainfall) remains stable for the same period (Fig. 1). The findings in Fig. 3 help us piece this puzzle together. Note that although the frequency histograms for the two periods (1951 to 1970 and 1981 to 2000) have significant differences (fig. S2), the mean rainfall during these periods is nearly identical at 5.75 mm and 5.69 mm, respectively. The heavy events (≥ 100 mm/day of rain) contribute about 6.4% to the seasonal mean, whereas moderate events (from 5 mm/day to < 100 mm/day) contribute about 85.8%. Although the relative contributions to the mean from these two classes do not balance in a given year, the contribution from the decreasing trend of moderate events is partially offset by that from increasing heavy rain events (7). Consequently, the seasonal total does not show any statistically significant change over longer time scales.

Previous attempts to detect trends in extreme rain events by using station data were inconclusive, probably because of the large year-to-year variability in Indian monsoon rainfall. To

assess the role of sampling and variability, we examined the number of heavy rain events over regions of increasing size (fig. S3). We find that for regions smaller than about 800 km by 800 km, it is difficult to find significant trends in heavy rain events. On the other hand, the whole of India cannot be taken as one unit to investigate such trends. The northeast and the west coast are regions of high mean (Fig. 1A) and high variability (fig. S1), and local orography has a strong influence on the rainfall over both regions. Therefore, trends in extreme rainfall due to a warming environment are difficult to discern in these regions.

In spite of considerable year-to-year variability, there are significant increases in the frequency and the intensity of extreme monsoon rain events in central India over the past 50 years. Although desirable for applications, it is difficult to detect signals of climate change in extreme rain events at individual stations; instead, as we show, one needs a sufficiently large area to discern a trend reliably. The observed trends suggest enhanced risks associated with extreme rainfall over India in the coming decades.

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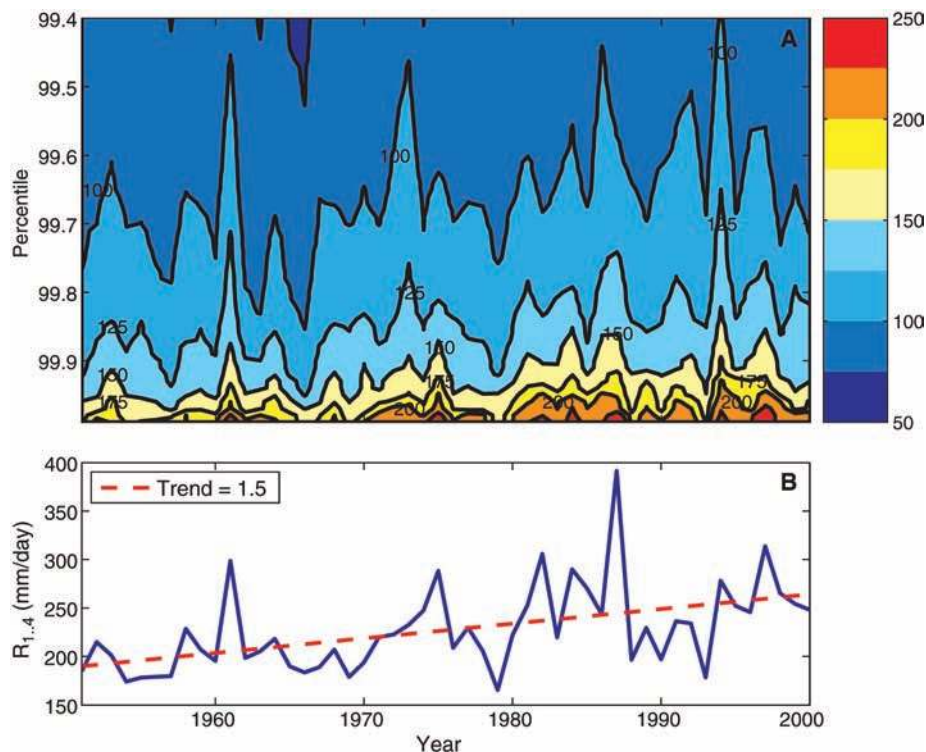


Fig. 4. Temporal variation (1951 to 2000) in (A) 99.4 to 99.99 percentiles of seasonal rainfall and (B) the mean rainfall of the four highest rain events every season ($R_{1..4}$). Color bar in (A) indicates rain intensity in mm/day. The statistical significance of the trend (dashed line) was calculated as in Fig. 2.

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Supporting Online Material

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Male Fertility and Sex Ratio at Birth in Red Deer

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Efforts to test sex ratio theory have focused mostly on females. However, when males possess traits that could enhance the reproductive success of sons, males would also benefit from the manipulation of the offspring sex ratio. We tested the prediction that more-fertile red deer males produce more sons. Our findings reveal that male fertility is positively related to the proportion of male offspring. We also show that there is a positive correlation between the percentage of morphologically normal spermatozoa (a main determinant of male fertility) and the proportion of male offspring. Thus, males may contribute significantly to biases in sex ratio at birth among mammals, creating the potential for conflicts of interest between males and females.

The Trivers and Willard hypothesis (1) for sex allocation predicts that parents should increase the production of the sex with the higher fitness benefit. This hypothesis has been applied most often to mothers, who have a strong influence on offspring quality through maternal care. It can also apply to any trait that parents transmit to offspring that has a differential effect on the reproductive success of sons and daughters. Thus, among birds, offspring sex ratios may be adjusted in relation to the attractiveness of the father, because sons will inherit large sexual ornaments and will achieve high reproductive success (2). However, it is assumed that such manipulation is under female control, because in birds females are the heterogametic sex.

The possibility that males may also facultatively adjust sex ratio has seldom been considered. In haplodiploid insects, the offspring sex depends on whether the ovum is fertilized or not, and males may constrain sex ratios because males with poor-quality ejaculates fail to fertilize the ova (3). In mammals, males are the heterogametic sex, and offspring sex is determined by whether an X- or Y-chromosome-bearing spermatozoon fertil-

izes the ovum. Thus, mammalian males may have more control over the mechanisms of sex determination than they do in other taxa. In mammals, male fertility may have a great influence on the reproductive success of sons.

Ungulates are good models to test sex ratio theory because they are sexually dimorphic in body size, variance in reproductive success is greater among males, and the reproductive success of sons is more strongly influenced by maternal investment. Early studies on red deer (*Cervus elaphus*) found support for the prediction that high-quality mothers should produce sons (4), but subsequent studies have generated inconsistent results (5). Our previous studies have shown that in natural populations of red deer, males differ markedly in their fertility rates, and more-fertile males have faster swimming sperm and a greater proportion of normal spermatozoa (6). Thus, male reproductive success may not

depend exclusively on body size, but also on the ability of males to fertilize females after copulation. Male fertility is advertised by antler size and complexity, so more-fertile males also have larger and more elaborate sexual characters, which may be inherited by their sons (7).

We tested the hypothesis that more-fertile red deer males produce more sons. The key challenge was to disentangle male and female effects by designing an experiment to retain the inter-male variation in fertility rates found in natural populations while minimizing differences between females (8). Thus, our experimental design was aimed at eliminating several female factors known to influence sex ratios: (i) We avoided the possibility that females may bias sex ratio in response to male quality by artificially inseminating females so that they had no direct experience with the males. (ii) We minimized differences in body condition by using a sample of females that were all in good physical condition, were kept under similar environmental conditions, and had access to an unlimited food supply. (iii) All females were inseminated at the same time in relation to ovulation, avoiding the confounding effects of insemination time. In contrast, by using sperm collected during the rut from males living in natural populations, we ensured a representative sample of the large degree of variation in male fertility previously described (6).

When the entire study sample is considered, a similar number of male and female offspring were produced (Table 1). However, among males, differences in fertility rates and in the proportion of male offspring were substantial. Male fertility rates ranged from 24 to 70%, and the proportion of male offspring ranged from 25 to 72% (Table 1).

Table 1. Descriptive statistics [mean, standard deviation (SD), and range] for male fertility rates, proportion of male offspring sired, percentage of normal sperm, sperm swimming-velocity parameters, and number of hinds inseminated per male ($n = 14$ red deer stags). VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity.

Parameters	Mean	SD	Range min–max
Fertility rate (%)	50.39	13.06	24–70
Proportion of male offspring	0.50	0.14	0.25–0.72
Morphologically normal spermatozoa (%)	80.07	8.78	65–95
VCL ($\mu\text{m/s}$)	126.87	28.48	85–163
VSL ($\mu\text{m/s}$)	67.86	27.31	28–111
VAP ($\mu\text{m/s}$)	88.74	26.52	53–122
Hinds inseminated per male	24.57	16.00	11–69

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Supporting Online Material for
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SUPPORTING ONLINE MATERIAL

Data Quality Control

The $1^\circ \times 1^\circ$ gridded data we use here has been developed by the India Meteorological Department (IMD) for the period 1951 to 2003. A total of 6329 stations (which include those maintained by IMD, individual state governments) cover the country, out of which 1803 stations have been used in developing the gridded product. These 1803 stations were chosen based on the constraint that they have at least 90% daily data availability during the 50-year period, so as to minimise temporal inconsistencies. Station data is interpolated to a grid using a weighted sum, which is a variant of a method adopted by the Global Precipitation Climatology Project (GPCP) (see 24 for details). Missing data are treated as is, with a flag of -99. However, given that the rainfall intensity at each $1^\circ \times 1^\circ$ grid-point is a weighted average from several stations, there is complete continuous coverage on all days during the 1951-2003 period. Consequently, no land point in the area under study has missing data.

The quality control procedure typically involves checking for coding and typing errors, and correcting them by referring to the original recording manuscripts. The number of stations has dropped significantly from 1800 (pre-1995) to 1000 (in 2000) to 250 (in 2003) owing to the delay in digitising and archiving (24) at IMD. Hence, we have used the data up to 2000 for our analysis. The number of stations in central India is about 600. The density of stations is not uniform throughout the region; in particular, the coverage over the eastern part of central India is sparse (approximately 50 stations in a $5^\circ \times 5^\circ$ area as compared to 150 stations in the other parts of central India). The major sources of systematic error are evaporation out of the gauge and droplets being blown across the gauge. Monthly rain from this gridded dataset has a correlation of 0.91 with VASClimo (Variability Analysis of Surface Climate Observations) $1^\circ \times 1^\circ$ gridded data from the German Weather Service (24).

The dataset can be obtained by contacting M. Rajeevan (rajeevan@imdpune.gov.in) or the National Climate Centre, IMD, Pune (ncc@imdpune.gov.in).

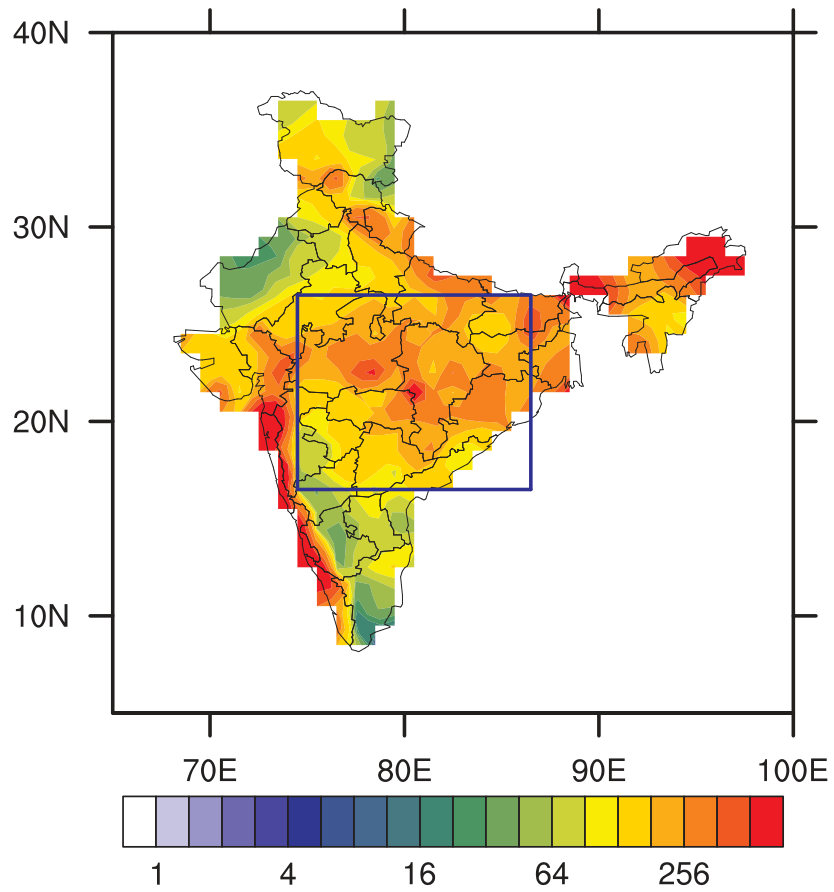


Fig. S1: Climatological mean variance of daily rainfall anomalies during June 1 - September 30 ($\text{mm}^2 / \text{day}^2$). The box indicates the CI region over which the daily rainfall variability can be considered to be spatially homogeneous.

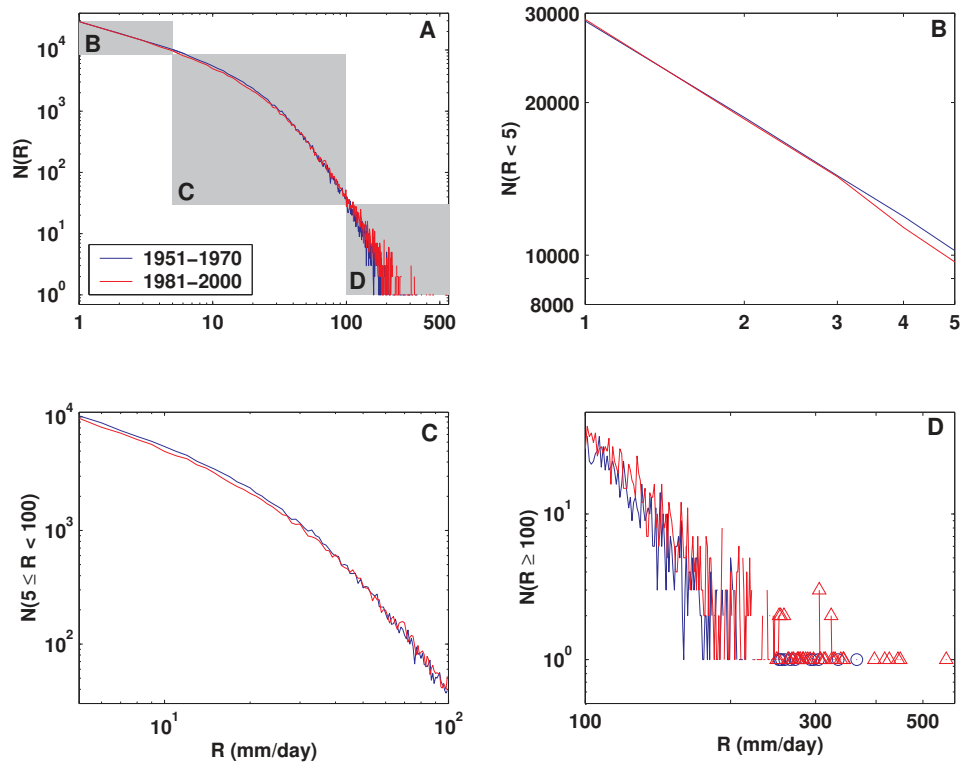


Fig. S2: (A) Frequency Histogram of daily rainfall over CI during summer monsoon for two periods, 1950-1970 and 1980-2000. The regions marked by the shaded rectangles in A are magnified in B, C, and D. For the sake of clarity, rain intensities larger than 250 mm/day have been shown by symbols (blue circles and red triangles) in panel (D).

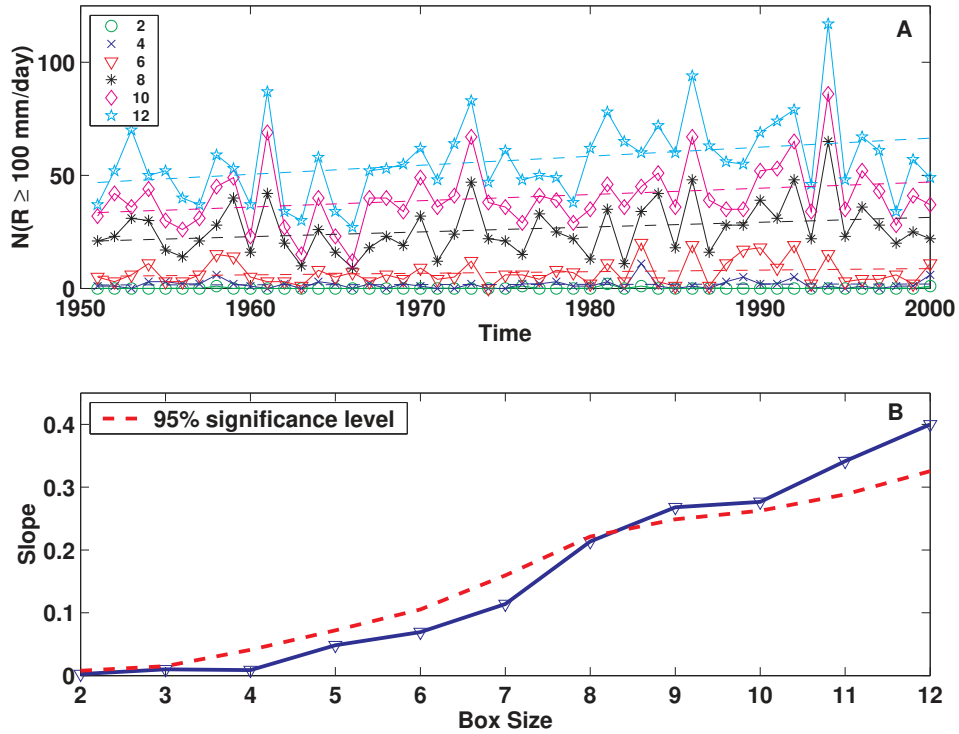


Fig. S3: (A) Temporal variation of the number of heavy rain events over increasingly bigger regions starting with a square box of size 2° at the lower left corner of CI to a box of size 12° . (B) The slope of the least-square fitted linear trends (blue) as a function of size of the region, together with the required slope to be significantly different from zero, at 0.05 significance level (red). The slope becomes significant for regions with size larger than $8^\circ \times 8^\circ$. Statistically significant trends are calculated as in Fig. 2.