Universitat Autònoma de Barcelona

On the role of nutrients, climate and anthropogenic impacts in spatio-temporal variability of forest productivity

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PhD Thesis

Marcos Fernández-Martínez

to be eligible for the Doctor degree

Supervised by:

Prof. Josep Peñuelas Reixach

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PhD in Terrestrial Ecology

Global Ecology Unit (CREAF-CSIC)

Center for Ecological Research and Forestry Applications

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PLUS ULTRA

Estello, fai-te clara,
car cerque moun camin.

Allà dellà de l'espai
he vist somriure una estrella
perduda en lo front del cel
com espiga en temps de sega,
com al pregon de l'afrau
una efímera lluerna.

—Estrelleta —jo li he dit—,
de la mar cerúlea gemma,
de les flors de l'alt verger
series tu la darrera?

—No só la darrera, no,
no só més que una llanterna
de la porta del jardí
que creies tu la frontera.

És sols lo començament
lo que prenies per terme.
L'univers és infinit,
pertot acaba i comença,
i ençà, enllà, amunt i avall,
la immensitat és oberta,
i aon tu veus lo desert
eixams de mons formigueguen.

Dels camins de l'infinit
són los mons la polsinera
que puja i baixa a sos peus
quan Jehovà s'hi passeja.

Al cel, Ms Jacint Verdaguer (190

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Abstract

Forests are among the most important terrestrial ecosystems in terms of photosynthesis and carbon sequestration because they accumulate and sequester large amounts of carbon, both as living tissues and as soil organic matter. Forests also provide large resource pulses through fruit crops that may present cascading effects through the food web of the ecosystem.

The main objective of the thesis was to characterize the structure and functioning of forests and to quantify ecosystemic productivity. We focused our research on understanding the main controls of carbon balance and fruit production in forest ecosystems. In this thesis, we try to disentangle the effects of nutrient availability, stand characteristics, climate, weather variability and anthropogenic impacts on spatio-temporal variability in forest productivity.

We found that stand age, water availability, and length of the warm period were the main factors controlling forest carbon stocks, fluxes and resource-use efficiencies. Standing biomass and carbon fluxes were strongly correlated to each other at the global scale and both were controlled by climate (mainly water availability and temperature) and stand characteristics such as the age of the stand or leaf type. However, carbon balance of the ecosystem strongly depended on the nutrient availability. We found that, nutrient-rich forests sequester 33 ± 4% of photosynthesized carbon while nutrient-poor forests only sequester 6 ± 4% of it. At the ecosystem level, this effect of nutrient availability was independent of climate, stand age or the management of the stand. In addition, once the effect of stand age was removed the biomass-to-net primary production ratio of woody tissues (branches, stems, and coarse roots) was positively influenced by nutrient availability, but it had a negative effect on the fine root fraction. Regarding temporal variability of carbon fluxes, we found that increasing CO_2 has increased gross primary production and carbon sequestration, on average, by 1% annually from 1995 to 2011. We also found that

the reduction of sulphur deposition in Europe and the USA involves a higher recovery in ecosystem respiration than in gross primary production.

Regarding fruit production, we found that fruit production ranges from 10 to 40 g C m^{-2} y^{-1} and uses around 0.5 - 3% of the photosynthesized carbon in European forests. We also found that forests with higher foliar zinc and phosphorus concentrations, produced larger fruit crops and presented less irregularity in interannual fruit crop size. On the other hand, we found interannual fruit variability to be controlled by the interannual variability of the North Atlantic Oscillation, having a more prominent importance than local weather variables in predicting it. In *Quercus ilex* and *Quercus pubescens* stands we found that spring water deficit was the most relevant factor in explaining interannual variability in acorn production and that interannual differences in pollen production did not influence acorn crop size. Spring water deficit was also the main factor affecting synchrony in fruit production among forests. We also demonstrated that fruit production can be predicted using remotely sensed vegetation indices, such as the enhanced vegetation index (EVI). Our results suggested that fruit crop size in *Quercus ilex* was driven by a combination of two factors, i.e. good vegetation conditions during several months prior to fruit harvest, and wet weather during spring. Finally, we demonstrated the usefulness of the consecutive disparity index (D) and its advantages with respect to the coefficient of variation (CV). We found D index to be less dependent on the mean than the CV while also taking into account the autocorrelation of the time series.

The results of this thesis open the door to a wide range of new ideas and hypotheses worth to test in the near future. This thesis is also a good example of what the so called "*big data*" can offer to ecologists and environmental scientists.

General introduction

Terrestrial ecosystems are key components of the global carbon cycle. Since the 1960s, they have been sequestering, on average, 15–30% of the anthropogenic CO_2 emitted into the atmosphere (Canadell *et al.*, 2007; Le Quéré *et al.*, 2009). Forests are among the most important of the terrestrial ecosystems in terms of photosynthesis and carbon sequestration. Forest ecosystems accumulate and sequester large amounts of carbon, both as living tissues and as soil organic matter (Dixon *et al.*, 1994; Myneni *et al.*, 2001; Pan *et al.*, 2011). The increasing atmospheric CO_2 concentration and its influence on global climate (IPCC, 2007) highlight the necessity to better understand the mechanisms driving the global carbon cycle. Understanding how these ecosystems are structured and how they function is therefore of paramount importance for improving our knowledge of the global carbon cycle and for predicting future climate.

The carbon balance of forest ecosystems

Forests capture carbon dioxide through photosynthesis, called gross primary production (GPP) when referring to the ecosystem scale, and release it through the respiration of the ecosystem (Re) (Figure 1). GPP and Re are, under normal conditions, the largest carbon fluxes in forest ecosystems (Luyssaert *et al.*, 2007). The difference between carbon uptake and release is the net ecosystem production (NEP) and provides information about the carbon balance of the ecosystem. When positive, NEP indicates carbon sinks (GPP > Re) while when negative, it indicate carbon sources (GPP < Re). Because lateral exportation and importation of carbon as dissolved organic carbon (DOC) or as particulate organic carbon (POC) is usually quite low, lateral inputs and outputs are often neglected and therefore, the general equation reads: NEP = GPP – Re (Figure 1).

Net primary production (NPP) is the difference between GPP and autotrophic respiration (Ra). The allocation of GPP to the different compartments of the ecosystem (different fractions of the NPP) is not trivial, since it will strongly determine the residence time of carbon in the ecosystem (Luo *et al.*, 2003; Zhang *et al.*, 2010). A considerable fraction of the NPP is allocated into relatively labile material, such as foliage, fruits or fine roots. These components are the main responsible for heterotrophic respiration (Rh), which is mainly related to decomposers and animals. The sum of Ra and Rh equals Re.

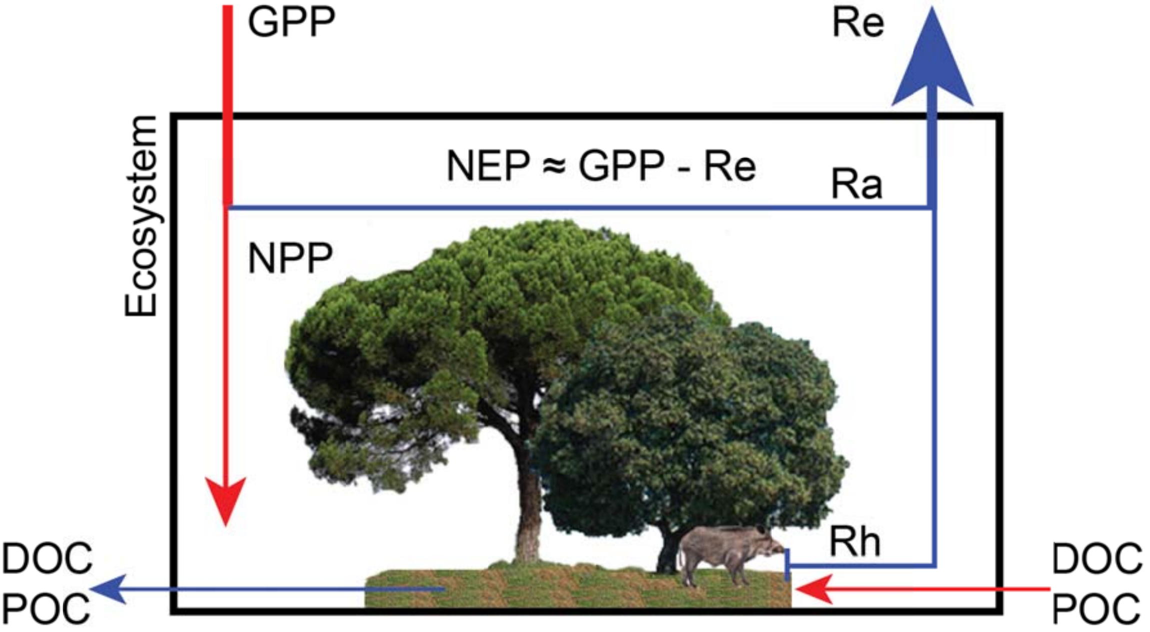

Figure 1: Scheme of the carbon balance of forest ecosystems. Acronyms are, as follow: gross primary production (GPP), ecosystem respiration (Re), net ecosystem production (NEP), net primary production (NPP), autotrophic respiration (Ra), heterotrophic respiration (Rh), dissolved organic carbon (DOC), particulate organic carbon (POC).

The carbon balance of forest ecosystems depends on a wide range of variables. On the one hand, it changes with the chronosequence of the ecosystem (Hougthon, 2009). At first, young ecosystems gradually increase GPP, NPP and NEP until a threshold is reached. Instead, the increase of Re is delayed in time with respect to NPP because decomposition (Rh) is slower than NPP. After the threshold of GPP and NPP is reached, NEP starts to decline (Figure 2) because respiration still increases. Theoretical assumptions of the carbon balance assumed that, with enough time, ecosystems should reach a steady state in which carbon inputs equals outputs and, therefore, NEP equals 0 averaged over years or decades (Hougthon, 2009). However, this assumption is highly unlikely to be met by all ecosystems. Carbon sequestration (positive NEP) must have taken place during large periods of time as far as we know about the existence of geologic oil and coal deposits. On the other hand, climate changes and multidecadal weather variability (such as those described by teleconnections (Parazoo *et al.*, 2015)), stochastic processes and perturbations (e.g., climate extremes, fires or pest outbreaks (Reichstein *et al.*, 2013)) or anthropogenic impacts (e.g., atmospheric deposition (Magnani *et al.*, 2007), increased atmospheric CO_2 (Keenan *et al.*, 2013)) can also alter the equilibrium. Furthermore, some authors have recently demonstrated that old-growth forests still act as carbon sinks (Luyssaert *et al.*, 2008). It is, therefore, far from clear that ecosystems should reach a steady state with time either theoretically or experimentally.

Spatially, carbon balance is highly dependent on climate and site characteristics (Luyssaert *et al.*, 2007, 2010). At the global scale, temperature, precipitation and radiation correlate well with ecosystem productivity (Nemani *et al.*, 2003), gross primary production and respiration (Beer *et al.*, 2010). Also, increasing atmospheric CO_2 concentrations have been suggested to increase forest productivity (Ainsworth & Long, 2005) and resource-use efficiency (Peñuelas *et al.*, 2011; Keenan *et al.*, 2013) through the so called CO_2 fertilization effect. However, different forests living under the same climate are likely to present differences among their carbon fluxes since different resource-use efficiencies (of carbon, nutrients, water or light) will determine the metabolism of the ecosystem. For example, some authors have pointed out that managed and nutrient-rich ecosystems produce biomass more efficiently compared to unmanaged and nutrient-poor ecosystems (Vicca *et al.*, 2012; Campioli *et al.*, 2015). However, up to now, little evidence has been presented demonstrating that nutrient availability alters carbon balance at the ecosystem level at the global scale. Similarly, poor evidences have been presented suggesting that the CO_2

fertilization effect occurs also in natural ecosystems and that this increase in photosynthesis or resource-use efficiency can affect the carbon balance of the ecosystem. In this thesis, we will make an effort to answer these particular questions.

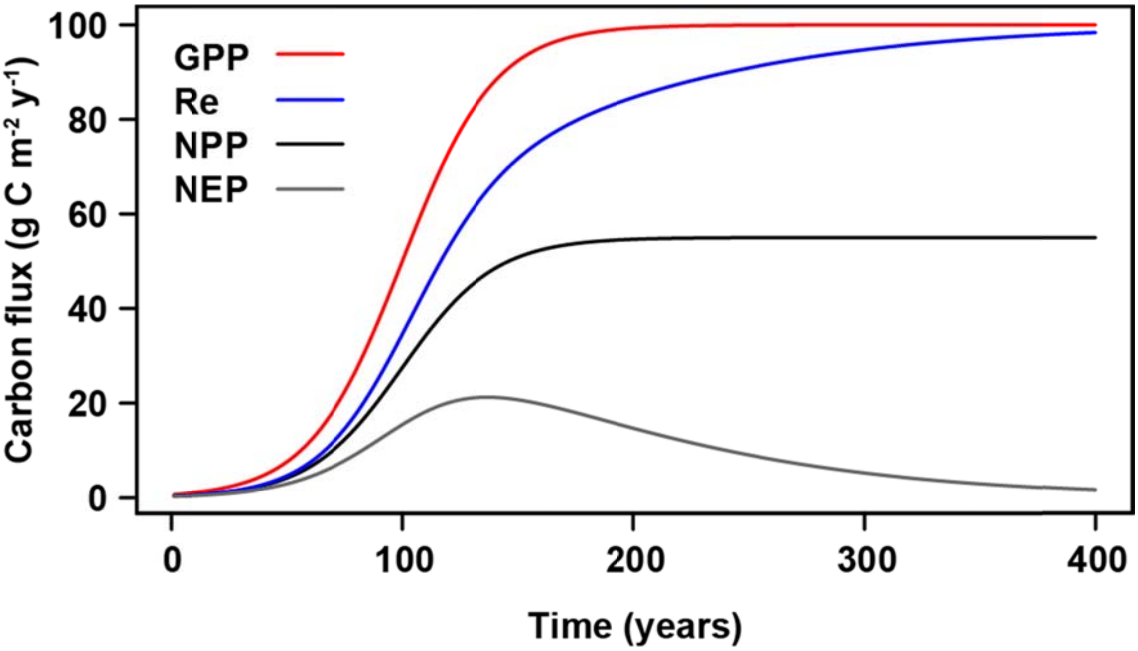

Figure 2: Theoretical evolution of the main carbon fluxes of an ecosystem.

Fruit production of forests

On the other hand, fruit or seed production, as a fraction of the NPP, is one of the most important life history traits in plants because it enables them to transmit their genes to the next generation (Fenner & Thompson, 2005). In an ecosystem, fruit production of the key species, can have significant effects on the entire community: by providing intermittent resource pulses (Yang *et al.*, 2008) fruit production regulates populations of consumers (Espelta *et al.*, 2008) potentially producing cascading effects on food webs (Ostfeld & Keesing, 2000) that can even affect human beings by the dispersion of illnesses like the lyme desease (Ostfeld, 1997).

Understanding the intricate patterns of fruit production in forests has received a lot of attention because of the *masting* phenomenon. The *masting* behaviour consists on the synchronization among individuals of a highly erratic production of fruits, combining years with huge fruit crops with years with zero or very few fruits produced (Silvertown, 1980; Kelly & Sork, 2002). Despite masting is a phenomenon that takes place at the population, the community or even at continental scales (Koenig & Knops, 1998, 2000) its genesis stands on two key aspects of fruit production originated at the individual level: i) the high interannual variability in fruit production and ii) the synchronization of fruit production among individuals. However, what triggers interannual variability and how plants synchronize their fruit productions is still mostly unknown.

Two main groups of hypotheses try to explain interannual variability in fruit production. On the one hand, mechanistic hypotheses such as the *resource matching* hypothesis (Norton & Kelly, 1988; Sork *et al.*, 1993; Kelly & Sork, 2002) suggesting that trees produce fruits as a direct response of the available resources, or the *resource accumulation* hypothesis (Isagi *et al.*, 1997), which suggests that trees accumulate resources (do not produce seeds) until the threshold for large crops is reached. On the other hand, hypotheses like the *pollination efficiency* (Norton & Kelly, 1988; Smith *et al.*, 1990; Koenig & Ashley, 2003), which states that synchronized and intermitent flowering increases pollination success in wind pollinated species, or the *predation satiation,* which suggest that starving and satiating fruit consumers by irregular fruit production is a selective trait that improves fitness (Silvertown, 1980; Espelta *et al.*, 2008) are based on the benefits of the *economies of scale*. The *economy of scale* hypotheses have been considered to be one of the most prominent explanations supporting masting as a selective response (Kelly & Sork, 2002).

Similarly to interannual variability, synchrony in fruit production has also been explained by different hypotheses. On the one hand, the Moran effect (Moran, 1953; Ranta *et al.*, 1997), hypothesizes that synchrony in fruit production is controlled by synchrony in weather conditions, which are used by trees either as cues to divert resources into reproduction or, mechanistically, producing more fruits when weather is favourable for acquiring more resources. Since weather conditions can also be spatially synchronous (Koenig, 2002), the Moran effect has also been hypothesised to be a potential synchronizer of fruit production over large geographic areas (Koenig & Knops, 2013). On the other hand, the *pollen coupling* hypothesis (Satake & Iwasa, 2002) suggests that the available pollen from neighbour trees can generate synchrony in fruit production among individuals by interacting with available resources for trees (i.e., a large maturation of female flowers after a massive pollination event (when enough pollen is available) would deplete the resources of all trees at a time, leading to the synchronization of fruit production within the population).

Despite the large bibliography focusing on interannual variability in fruit production, little efforts have been done up to now to characterize the spatial variability of forest fruit production. Hence, in his thesis we will focus on the spatio-temporal variability in fruit production while trying to relate our results to the existing *masting* hypotheses.

Objectives of the thesis

The main objective of the thesis was to elucidate the main controls of forest structure and function and the drivers of their temporal variability. In order to accomplish that, we divided the thesis in 9 chapters aggregated in three blocks. In the first block we focus on ecosystemic productivity of forests, with special emphasis on carbon balance. In the second block, we focus our efforts on understanding the main drivers of fruit production of forests and, finally, in the third block we present new methodologies that we have developed and already used in the thesis. In order to answer the main question of the thesis we posed the following specific aims in each of the chapters of the thesis.

In Chapter 1 we aimed to provide a synthesis of what forest ecosystems are and how they function providing: a) an accurate and detailed description of the biomass stocks, annual carbon fluxes, and resource-use efficiencies of forest ecosystems in different biomes on a global scale based on field and remote-sensing data, and b) a determination of the main drivers of the spatial variability in biomass stocks and in their distribution among ecosystem compartments, the annual carbon fluxes, and the resource-use efficiencies.

In Chapter 2, we aimed to test whether nutrient availability was related to higher carbon sequestration in forest ecosystems hypothesizing that both NEP and CUEe would increase with increasing nutrient availability.

In Chapter 3, the aim of the study was to calculate the B:NPPs of five compartments (foliage, branches, stems, coarse roots, and fine roots) of forest ecosystems around the world and to explore the endogenous (stand age and leaf habit and type) and exogenous (climate and management) factors that control them. We also particularly investigated the role of nutrient availability as a likely control of B:NPP, hypothesizing that higher nutrient availability would be correlated positively with higher B:NPP in aboveground compartments and negatively in belowground compartments.

In Chapter 4, we tested the hypothesis that GPP, Re and NEP have been accelerated during the last two decades by the increased temperature and CO_2, and the recovery from high loads of sulphur deposition in Europe and North America. This chapter ends the first block of the thesis.

In Chapter 5, we aimed to characterize fruit production and its spatio-temporal behaviour for several European tree species, while also disentangling the species-specific variability from the effect of productivity, foliar nutrient concentrations, climate and atmospheric deposition of nitrogen and sulphur.

In Chapter 6, we aimed to test whether seasonal NAO indices are useful to predict interannual variability and synchrony in fruit production by constructing statistical models including local weather (temperature and precipitation) variables and NAO indices for previous autumn, winter, spring and summer seasons, using 76 forests of *Abies alba*, *Fagus* *sylvatica*, *Picea abies*, *Pseudotsuga menziesii*, *Quercus petraea* and *Quercus robur* distributed across central Europe. We aimed to discuss the possible contribution of the different *masting* hypotheses on interannual variability and synchrony in fruit production with the results provided by models.

In Chapter 7, we aimed to test whether pollen availability or resources are the major forces driving *masting* in two Mediterranean oaks (*Quercus ilex* and *Quercus pubescens*), and to evaluate whether interannual differences in the flowering phenology and weather may also influence this process.

In Chapter 8, we aimed to explore whether the pattern of seed production in Mediterranean oak (*Quercus* spp.) forests could be governed by the temporal dynamics of tree canopies, assessed by means of remote sensing indices. We hypothesised that vegetation changes, detected using EVI, represent a reliable proxy of the tree resources available for seed production and that it should, therefore, be correlated to fruit crop size. This chapter ends the second block of the thesis.

In Chapter 9, we aimed to demonstrate the advantages of D over the CV for capturing temporal variability in different ecological time series and for different types of studies, focusing the analyses on interannual and intra-annual variability and on the early warning signals in time series approaching an abrupt shift. This chapter is the only one included in the third block of the thesis.

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Block 1

Production and carbon balance of forest ecosystems

Chapter 1

Spatial variability and controls over biomass stocks, carbon fluxes, and resource-use efficiencies across forest ecosystems

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Abstract

We aimed to discern the distribution and controls of plant biomass, carbon fluxes, and resource-use efficiencies of forest ecosystems ranging from boreal to tropical forests. We analysed a global forest database containing estimates of stand biomass and carbon fluxes (400 and 111 sites, respectively) from which we calculated resource-use efficiencies (biomass production, carbon sequestration, light, and water-use efficiencies). We used the WorldClim climatic database and remote sensing data derived from the Moderate Resolution Imaging Spectroradiometer to analyse climatic controls of ecosystem functioning. The influences of forest type, stand age, management, and nitrogen deposition were also explored. Tropical forests exhibited the largest gross carbon fluxes (photosynthesis and ecosystem respiration), but rather low net ecosystem production, which peaks in temperate forests. Stand age, water availability, and length of the warm period were the main factors controlling forest structure (biomass) and functionality (carbon fluxes and efficiencies). The interaction between temperature and precipitation was the main climatic driver of gross primary production and ecosystem respiration. The mean resource-use efficiency varied little among biomes. The spatial variability of biomass stocks and their distribution among ecosystem compartments were strongly correlated with the variability in carbon fluxes, and both were strongly controlled by climate (water availability, temperature) and stand characteristics (age, type of leaf). Gross primary production and ecosystem respiration were strongly correlated with mean annual temperature and precipitation only when precipitation and temperature were not limiting factors. Finally, our results suggest a global convergence in mean resource-use efficiencies.

Commonly used abbreviations:

Biomass and carbon-flux variables

LAI: leaf area index [m^2 m^{-2}]
SLA: specific leaf area [m^2 kg^{-1}]
GPP: gross primary production [g C m^{-2} $year^{-1}$]
Re: ecosystem respiration [g C m^{-2} $year^{-1}$]
NEP: net ecosystem production [g C m^{-2} $year^{-1}$]
TBP: total biomass production [g C m^{-2} $year^{-1}$]
ABP: aboveground biomass production [g C m^{-2} $year^{-1}$]
FNPP: foliage net primary production [g C m^{-2} $year^{-1}$]
WNPP: wood net primary production [g C m^{-2} $year^{-1}$]
BBP: belowground biomass production [g C m^{-2} $year^{-1}$]
ABP%: ABP to GPP ratio [%]
FNPP%: FNPP to GPP ratio [%]
WNPP%: WNPP to GPP ratio [%]
BBP%: BBP to GPP ratio [%]

Efficiency variables

CUEe: carbon use efficiency at the ecosystemic level [%]
BPE: biomass production efficiency [%]
LUE: light-use efficiency [g C MJ^{-1}]
$LUE\%_{APAR}$: light-use efficiency relative to absorbed PAR [%]
$LUE\%_{PAR}$: light-use efficiency relative to incident PAR [%]
PAR: photosynthetically active radiation [M J m^{-2}]
$LUE\%_{TRad}$: light-use efficiency relative to total incident radiation [%]
WUE: water-use efficiency [g C L^{-1}]

Climatic variables

AET: actual evapotranspiration [mm $year^{-1}$]
PET: potential evapotranspiration [mm $year^{-1}$]
WD: water deficit [%]
MAT: mean annual temperature [°C]
MAP: mean annual precipitation [mm $year^{-1}$]

1. Introduction

The increasing atmospheric CO_2 concentration and its influence on global climate (IPCC, 2007) highlight the necessity to better understand the mechanisms driving the global carbon cycle. Forest ecosystems sequester and store large amounts of carbon, both as living biomass and as dead organic matter (Dixon *et al.*, 1994). Understanding how these ecosystems are structured and how they function is therefore of paramount importance for improving our knowledge of the global carbon cycle and for predicting future climate.

Previous studies (Margalef, 1974; Dixon *et al.*, 1994; Malhi *et al.*, 1999; Gower *et al.*, 2001; Litton *et al.*, 2007; Luyssaert *et al.*, 2007; Keith *et al.*, 2009; Pan *et al.*, 2011) have reported estimates of the stocks and distribution of biomass among different ecosystemic compartments, carbon fluxes, or resource-use efficiencies for different regions and taxa, but most of the combined analyses of biomass distributions, carbon fluxes and resource-use efficiencies were based on limited subsets of climate space. Continuous efforts of the research community have rapidly increased data availability, and following methodological harmonisation has provided these data to be compared more easily.

Despite extensive literature on controls of resource-use efficiencies (Garbulsky et al., 2010; Gu et al., 2002; Jenkins et al., 2007; Landsberg and Waring, 1997; Wang et al., 2003 for light or radiation use efficiency and Field et al. 1983; Huxman et al. 2004; Yu et al. 2008; Troch et al. 2009; Lu and Zhuang 2010; Peñuelas et al. 2011 for water- or precipitation-use efficiency), differences in resource-use efficiency among biomes or forest types is seldom explicitly reported (but see Goetz and Prince, 1999 for LUE and Yu et al., 2008 for WUE), or the available data are insufficient for generalising the results to the entire biome or forest type under study. In this study, we will consider the resource-use efficiency for light (LUE), water (WUE), biomass production (BPE) and short term carbon sequestration at the ecosystemic level (CUEe).

Studies of climatic control concerning forest production or functioning have frequently tested the influence of temperature, precipitation, drought indices, or radiation (Kato and Tang, 2008; Law et al., 2002; Luyssaert et al., 2007; Magnani et al., 2007; Valentini et al., 2000), although some other variables such as thermal amplitude, seasonality of precipitation, and actual or potential evapotranspiration may be key determinants of forest functioning (Garbulsky *et al.*, 2010). In addition, the relative contributions to ecosystem structure and functioning of climate and forest characteristics such as stand age (Goulden et al., 2011; Magnani et al., 2007; Vicca et al., 2012), management (Luyssaert *et al.*, 2007; Vicca *et al.*, 2012), or nitrogen deposition (De Vries et al., 2009; Luyssaert et al., 2010; Magnani et al., 2007) are still debated.

In this study, we have updated a global database (Luyssaert *et al.*, 2007) of forest carbon pools and fluxes and have extended this database with 45 new forests and data up to 2010, remote-sensing observations of actual evapotranspiration (AET) and the absorbed fraction of photosynthetically active radiation (fPAR) to calculate water-use and light-use efficiencies (WUE, LUE). Our aim was to update the analysis done by (Luyssaert *et al.*, 2007) and to extend it with: i) an accurate and detailed description of the biomass stocks, annual carbon fluxes, and resource-use efficiencies of forest ecosystems in different biomes on a global scale based on field and remote-sensing data, and ii) a determination of the main drivers of the spatial variability in biomass stocks and in their distribution among ecosystem compartments, the annual carbon fluxes, and the resource-use efficiencies.

2. Materials and Methods

2.1. Collection and selection of data

2.1.1 Global forest database

To characterise forest ecosystems, we used a publicly available database of global forests (Luyssaert *et al.*, 2007). This database contains measurements, and in some cases their uncertainties, for several structural, functional, and bioclimatic characteristics and a compilation of simulated data such as, for example, nitrogen deposition for 558 forests around the world. To characterise forest ecosystems, we extracted from this database, for each forest, the following variables (whenever available): i) maximum LAI, ii) stand biomass of living trees, comprising estimates of aboveground biomass (typically distinguishing between woody and foliar biomass) and of fine and coarse roots, iii) mean annual carbon fluxes, comprising estimates of GPP, Re, NEP (derived from eddy covariance towers), TBP, aboveground and belowground biomass production and NPP of wood and leaves, and iv) APAR (absorbed photosynthetically active radiation) used to calculate LUE (GPP:APAR; see section 2.1.4).

We also extracted several variables to use as predictors of the stand characteristics: i) nitrogen deposition derived from interpolated gridded maps based on ground observations (Holland et al., 2005)) and model simulations (Galloway et al. 2004), ii) stand age, iii) leaf habit (evergreen or deciduous) and type (needleleaved or broadleaved), iv) management (managed, unmanaged, disturbed), and v) biome (boreal, temperate, Mediterranean, and tropical). The methodologies used to estimate all the variables within the database were reported by (Luyssaert *et al.*, 2007). We selected these predictor variables since they have been previously suggested to influence carbon balance and functioning in terrestrial ecosystems (De Vries et al., 2009; Luyssaert et al., 2007; Janssens et al., 2010; Magnani et al., 2007; Peñuelas et al., 2012; Vicca et al., 2012).

2.1.2 Geographical scale and forest types

All continents and biomes were represented in our database and analyses, but the available forests were distributed mostly around Europe and North America (Figure S1.1 and Figure S1.2 in Supporting Information), containing forests belonging to four different biomes (boreal [N=102], temperate [N=252], Mediterranean [N=11], and tropical [N=35] from which 10 were semi-arid forests and 25 were humid). Boreal and especially temperate forests were well represented in our database, but tropical and especially Mediterranean forests were underrepresented. The southern hemisphere was also poorly represented. The database contained evergreen, deciduous, needleleaved, broadleaved, and mixed (broadleaved-needleleaved) forests. Both leaf type and habit were well represented, but mixed forests were clearly in a minority.

2.1.3 WorldClim database

We used the WorldClim database (Hijmans *et al.*, 2005) because, i) it has a high spatial resolution (30 arc seconds ~ 1 km at the equator), ii) it contains robust monthly climatic data derived from long time series (from 1950 to 2000), and iii) although only data for temperature and precipitation are available, the dataset provides several bioclimatic variables such as annual thermal amplitude or seasonality of temperature and precipitation.

2.1.4 MODIS time series

We downloaded the evapotranspiration time series (MOD16A2) from MODIS (Moderate Resolution Imaging Spectroradiometer) for the period between January 1, 2000 and December 27, 2009 to obtain climatic surrogates of potential and actual evapotranspiration (PET and AET respectively, Mu et al. 2007) to use them as climatic predictors and to calculate WUE (GPP divided by AET). The resolution of the data was 9 km^2 around the central coordinates of the forest. We selected such a coarse resolution because of the poor resolution of the coordinates provided by the database.

To calculate LUE relative to total PAR (GPP:PAR), we downloaded the fPAR (absorbed fraction of PAR) time series (MOD15A2) from MODIS (Fritsch *et al.*, 2012) for the period between February 18, 2000 and December 27, 2011 with a resolution of 9 km^2 around the central coordinates of the forest. Once we calculated the average fPAR for each forest during the entire time series, we calculated total PAR using the following equation: APAR=fPAR*PAR, using APAR from the global forest database (see above).

2.2. Data analyses

2.2.1 Studied forest variables

To describe the characteristics and analyse the functioning of forest ecosystems, we separated the variables under study into three groups: i) stand biomass, ii) annual carbon flux, and iii) resource-use efficiency. The biomass variables were total, aboveground, belowground (coarse + fine roots), woody (trunk + branches), and foliar biomass. We also included the maximum LAI, a surrogate of SLA (maximum LAI · foliar biomass^{-1}), and the percentage of foliar, woody, and belowground biomass relative to total biomass. The variables of carbon flux were GPP, TBP, aboveground, belowground, woody and foliar biomass production (~NPP), Re, NEP, and the percentage of GPP partitioned into aboveground (ABP%), belowground (BBP%), woody (WNPP%), and foliar NPP (FNPP%).

Finally, the variables of resource-use efficiency were i) carbon-use efficiency at the ecosystemic level (CUEe), defined as the percentage of NEP to GPP, ii) biomass production efficiency (TBP:GPP, see Vicca et al., 2012) in percentage (BPE), iii) light-use efficiency (LUE), as the ratio of GPP to APAR and, iv) water-use efficiency (WUE), as the ratio of GPP to AET. We additionally calculated LUE expressed as the percentage of the energy converted into organic matter (glucose) relative to the amount received as APAR, PAR, and total incident radiation, considering that: i) 1 g of glucose equals 4.1 kcal (Margalef, 1974), ii) APAR equals PAR*fPAR, and iii) PAR represents 45% of the total incident radiation.

2.2.2 Climatic predictors

We extracted mean monthly and annual values for temperature (MAT) and precipitation (MAP) from the WorldClim database. We also extracted two key bioclimatic variables: annual thermal amplitude (mean maximum minus mean minimum temperature for the year, which provides information not only about the latitude of the forest but also about its continentality and elevation) and seasonality of precipitation (measured as the coefficient of variation of precipitation among months), which provides information about seasonal differences in the amount of precipitation.

We calculated the percentage of water deficit (WD) as an indicator of the intensity of water stress that the forests must tolerate. We calculated it from MODIS data as a percentage (WD = (1 – [AET/PET])*100) rather than as an absolute value (Stephenson, 1998) due to the large climatic gradient included in our data that might lead to different sensitivities of the biological variables to absolute values of water deficit in forests from different biomes (e.g. a change of 50 mm^{-1} $year^{-1}$ in a boreal forest with a MAP = 300 mm $year^{-1}$ may have a greater influence than in a tropical forest with MAP = 2500 mm $year^{-1}$). We also calculated the length of the warm period, considered as the number of months whose mean temperature was above 5 °C. We then extracted mean temperature, precipitation, potential and actual evapotranspiration, and water deficit during the warm period.

Summarising, we used 13 climatic predictor variables: PET, AET, WD, MAT, MAP (these five calculated both on an annual basis and for the warm period only), length of the warm period, annual thermal amplitude, and seasonality of precipitation. Correlations among the climatic variables are shown in Table S1.1, Supporting information.

2.2.3 Statistical analyses

To describe forest ecosystems, we first computed the averages of biomass, carbon flux and efficiency variables grouping forests according to biome and leaf type. As data were usually not normally distributed, we opted to perform bootstrapped (n=1000) weighted means and to use the bias-corrected and accelerated 95% confidence intervals (Efron, 1987) to test for differences between groups. We weighted cases according to the inverse of the uncertainty, except for biomass variables for which uncertainty was not reported in the database. We subsequently explored the possible relationships among different variables of biomass and flux via Pearson correlation analysis. We correlated the same dependent variable up to 10 times, so we controlled the rate of false discovery with Bonferroni corrections.

We performed stepwise forward-regression models to correlate the variables of biomass, carbon flux, and resource-use efficiency with climate, management, leaf habit and type, stand age, and nitrogen deposition. Predictor covariates were entered twice in the models, without transformation and transformed into the natural logarithm, to check for possible nonlinearities. We excluded the variables from the models when presenting high collinearity (Variance Inflation Factor [VIF] > 5).

Because controls of GPP, Re, and NEP are of paramount importance in the carbon balance of ecosystems, we closely examined the relationships of these three variables of carbon flux with MAT and MAP. We thus classified forests according to MAT and MAP, defining thresholds based on the median of the distribution of our data. Forests with MAP ≥827 mm year^{-1} were considered *wet* forests, and forests with MAP <827 mm year^{-1} were classified as *dry*. Similarly, forests with MAT ≥8 °C were considered *warm* forests, whereas forests with MAT <8 °C were classified as *cold*. Hence, forests were classified according to two-factor variables, each with two levels (wet/dry, warm/cold). This classification allowed us to test whether the relationships of the different variables of carbon flux with MAT depended on the *wetness* and whether the relationships between carbon fluxes and MAP depended on the *warmness*. To this end, we constructed multivariate generalised linear models (MGLM), including the interaction between *wetness* or *warmness* and MAT or MAP. Significant interactions would indicate changes in the relationships between carbon fluxes and climate according to *wetness* or *warmness*.

Finally, to analyse the direct, the indirect and the total effects of climate and nitrogen deposition on GPP, Re and NEP, we performed a path analysis using AET, MAT and nitrogen deposition as exogenous variables and GPP, Re and NEP as endogenous variables. The saturated model comprised all possible paths between exogenous and endogenous variables, a path from GPP to Re and a path from GPP and Re to NEP. We achieved the minimum adequate model by deleting, from the saturated model, those paths that were not found significant.

3. Results

3.1. Global variation in biomass, carbon fluxes and efficiency

3.1.1. Characterisation of biomass stocks

According to the bootstrapped 95% confidence intervals (which indicate significant differences when they do not overlap), the distribution of biomass among compartments revealed large differences among biomes (Figure 1.1 and Table S1.2, Supporting information) despite comprising forests of similar age, around 80 years old (Factorial ANOVA test: age ~ biome * leaf type, $P = 0.95$). Tropical and temperate forests presented the largest amounts of wood, aboveground biomass, and total-stand biomass, with values above 10 000 g $C{\cdot}m^{-2}$, while Mediterranean broadleaved forests exhibited the lowest values (Figure 1.1 and Table S1.2, Supporting information). Belowground biomass was lowest for boreal forests. A statistically significant difference was detected only for boreal versus temperate forests, although tropical forests also revealed a clear (and borderline significant) tendency of higher belowground biomass compared to boreal forests (Figure 1.1 and Table S1.2, Supporting information).

The distribution of the biomass stocks among different plant organs also differed among biomes. In both the boreal and temperate zone, needleleaved forests presented higher percentages of foliar biomass than broadleaved forests (above 5.5% and below 2.5%, respectively, Table S1.2, Supporting information). However, the percentage of biomass represented by wood, about 70% in most cases, did not differ between biomes or type of leaf. Finally, almost half of the biomass in Mediterranean broadleaved forests occurred belowground, whereas root biomass did not exceed 25% in all other forest types (Figure 1.1 and Table S1.2, Supporting information).

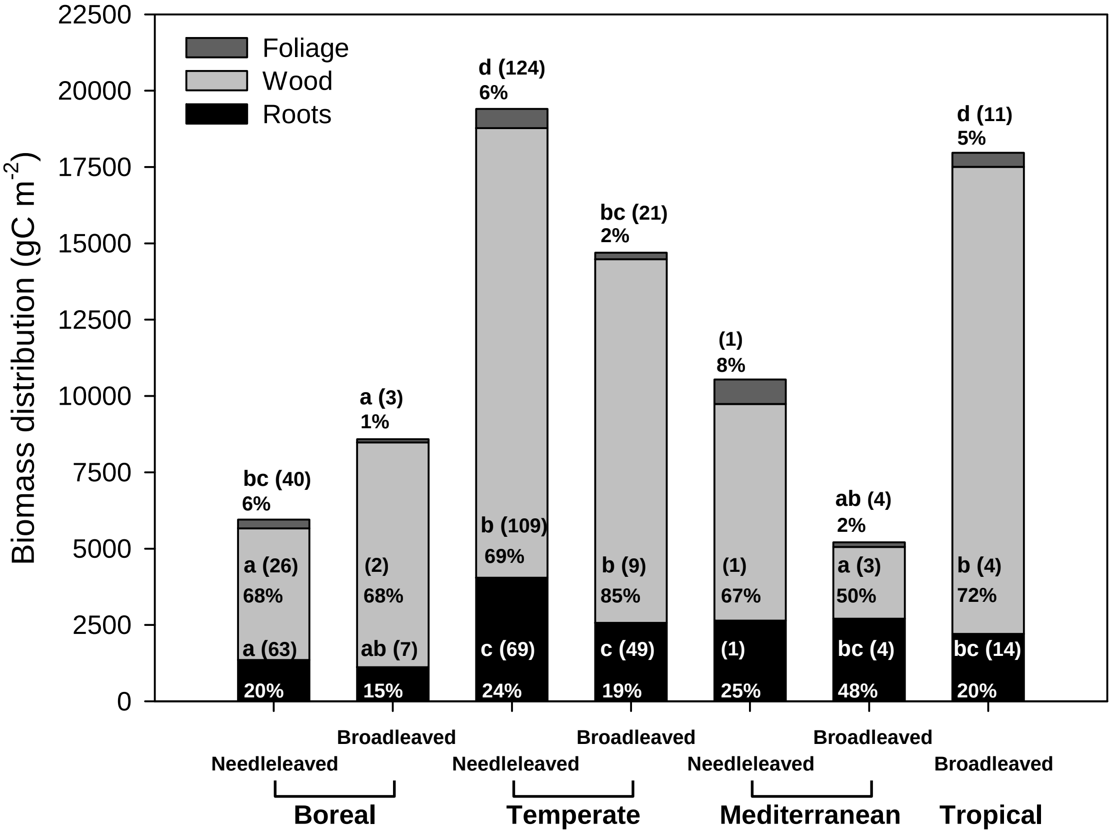


Figure 1.1: Biomass distribution among compartments in different forest types and biomes. It is also shown the percentage of biomass that each compartment represents in relation to the total biomass. Letters indicate significant differences among groups at the 0.05 level (see Table S1.3 in Supporting information for average values and confidence intervals) followed by the number of replicates (between brackets). Missing letters indicate that the comparison was not possible due to the lack of replicates.

The largest foliar biomass was found in temperate needleleaved and tropical broadleaved forests. The type of leaf was a crucial determinant of foliar biomass, because needleleaved forests had about 2.8 times more foliar biomass than did broadleaved forests across boreal and temperate biomes. Maximum LAI, however, did not significantly differ between leaf types within a single biome. The maximum values were found in temperate forests (LAI > 5), whereas the lowest were reported in Mediterranean broadleaved forests (LAI < 2.7, Table S1.2, Supporting information). In broadleaved forests, the specific leaf area (SLA = LAI·foliar biomass^{-1}), decreased with decreasing latitude, ranging from about 36 in boreal forests to 14 m^2 kg^{-1} in the tropics. Needleleaved boreal forests showed the lowest SLA (12.6 ± 1.2 m^2 kg^{-1}, mean ± SE) and statistically differed from both boreal and temperate broadleaved forests (Table S1.2, Supporting information).

3.1.2. Distribution of carbon fluxes

All carbon fluxes and their partitioning variables revealed significant differences among biomes but not between needleleaved and broadleaved forests (Figures 1.2 and 1.3 and Table S1.3, Supporting information). Tropical forests presented the highest rates for all fluxes, except for NEP (Figures 1.2 and 1.3a); the highest amount of NEP was observed at mid-latitudes (temperate and Mediterranean forests). Remarkably, tropical forests had rates of FNPP as high as the rates of TBP in boreal forests (Figure 1.3a).

Boreal needleleaved forests partitioned about 6% of their carbon uptake (i.e. GPP) into the foliage compartment (FNPP), whereas temperate and tropical broadleaved forests partitioned almost 12% of GPP to foliage. Wood NPP ranged between 10% (boreal needleleaved and tropical broadleaved forests) and 16% (temperate broadleaved) of GPP, while roots received between 8% (tropical broadleaved) and 18% (temperate broadleaved) of total GPP (Figure 1.3b).

3.1.3. Resource-use efficiency of biomes

CUEe followed the same pattern as NEP, being highest for temperate and lowest for tropical forests (Figure 1.2, Table S1.4, Supporting information). On average, about 20% of GPP was sequestered (NEP) in temperate-forest ecosystems, whereas only 3.3 ± 2.8% of the GPP of tropical broadleaved forests was actually stored. In contrast, BPE did not significantly differ among biomes, ranging from 38 ± 3.6% to 54 ± 4.7% (Table S1.4, Supporting information).

LUE ranged from 2.0 ± 0.2 (in temperate and Mediterranean broadleaved forests) to 2.6 ± 0.1 g C MJ^{-1} (in temperate needleleaved forests), with the former being significantly lower than the latter. When transforming this efficiency into the percentage of energy used, we found that forest ecosystems used between 8.5 ± 0.7% and 11.1 ± 0.6% of the absorbed light energy, between 5.5 ± 0.6% and 7.6 ± 0.5% of the total incident PAR, and between 2.5 ± 0.3% and 3.4 ± 0.2% of the total

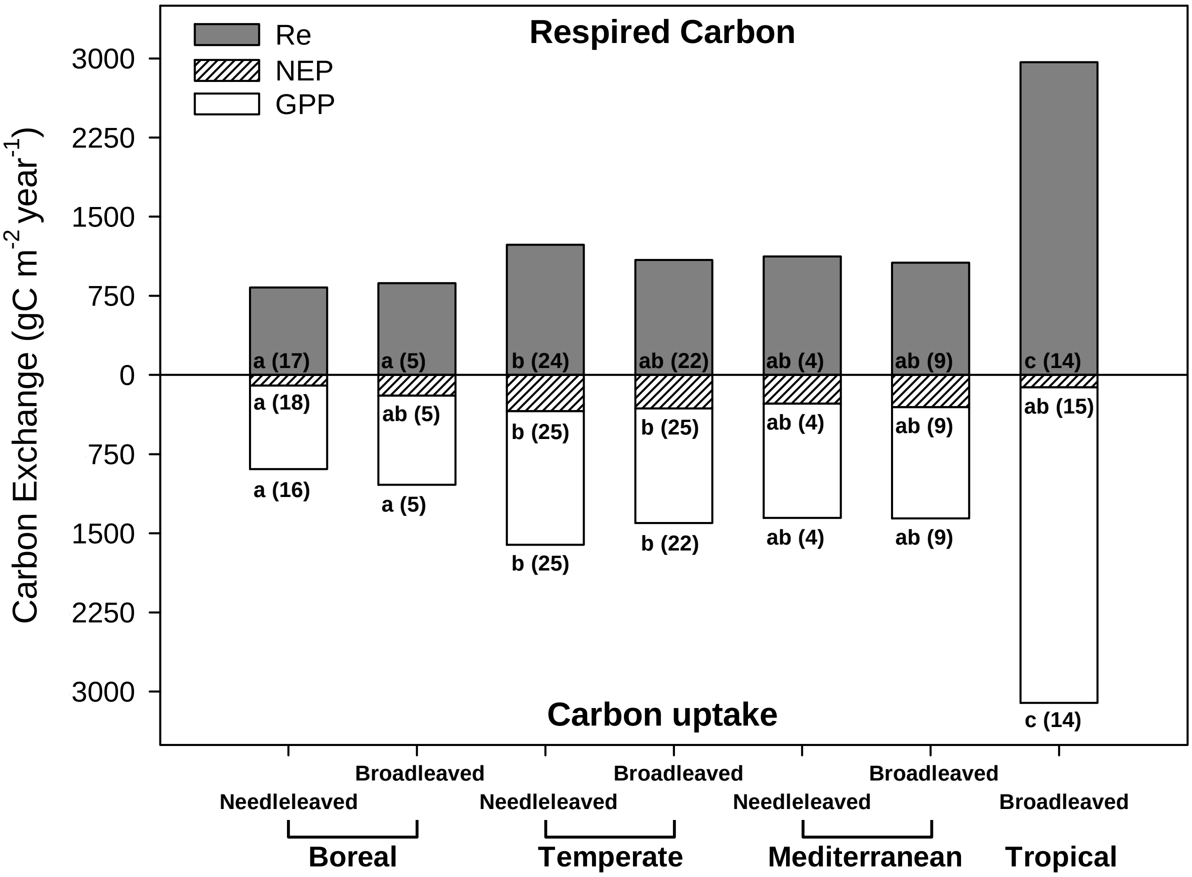


Figure 1.2: Mean values of carbon exchange between forest ecosystems and the atmosphere for different biomes. Letters indicate significant differences among groups at the 0.05 level (see Table S1.3 in Supporting information for average values and confidence intervals) followed by the number of replicates between brackets.

incident radiation (Table S1.4, Supporting information). Statistically, WUE did not differ among forest types due to high variability. On average, WUE ranged from 2.1 ± 0.2 to 3.1 ± 0.6 g C mm^{-1} in Mediterranean broadleaved and needleleaved forests, respectively.

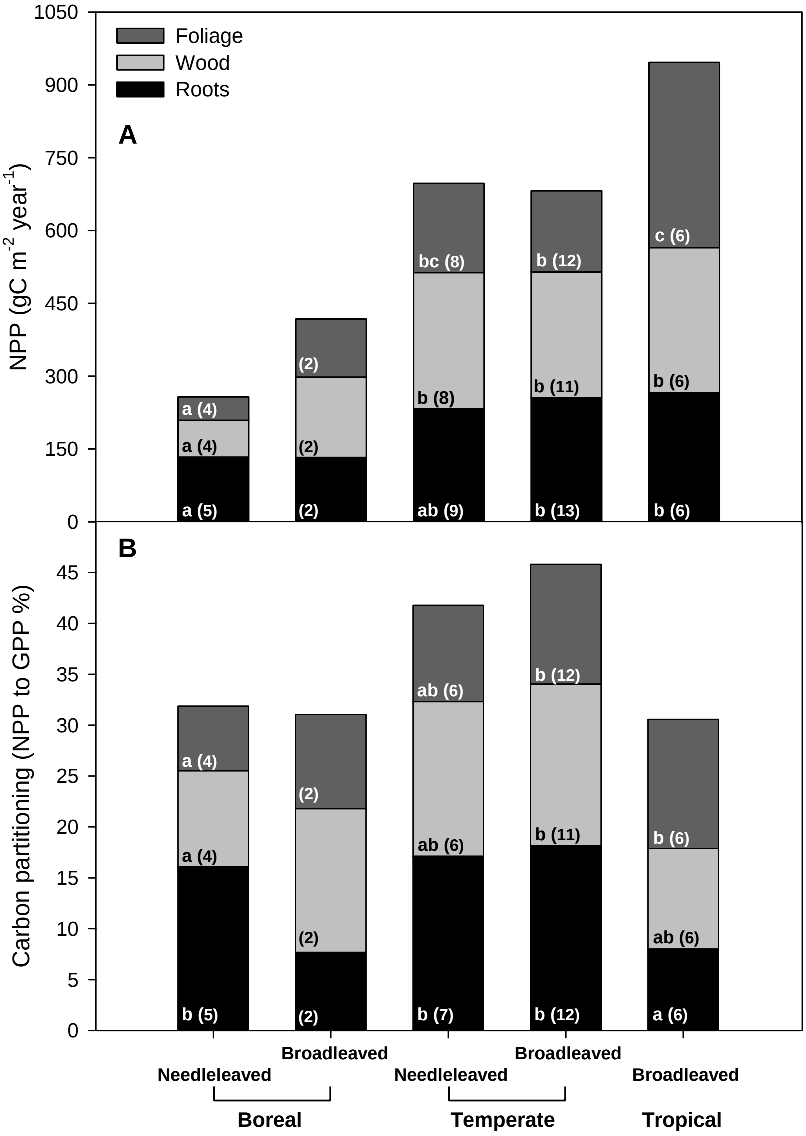


Figure 1.3: a) Net primary production (NPP) distribution (FNPP, WNPP, BBP) and b) partitioning of gross primary production (GPP) among compartments (FNPP%, WNPP%, BBP%) in different forest types and biomes. In graphs a and b, the total heights of the bars represent TBP and BPE, respectively (only forests with foliar, wood and root measurements available). Letters indicate significant differences among groups at the 0.05 level (see Table S1.3 in Supporting information for average values and confidence intervals) followed by the number of replicates between brackets. Missing letters indicate that the comparison was not possible due to the lack of replicates.

3.2. Correlations with biomass stocks, carbon fluxes, and efficiencies

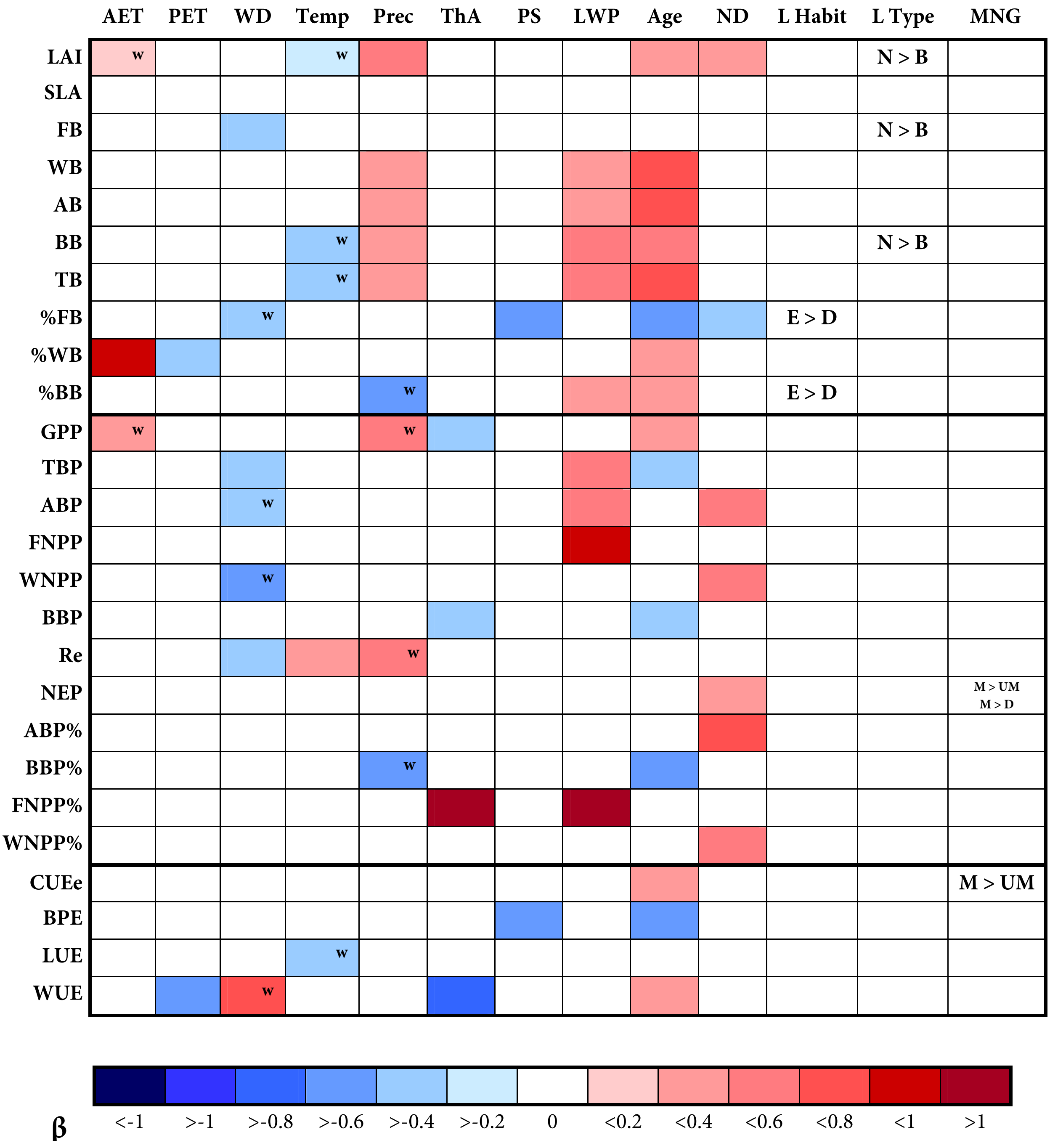
3.2.1. Biomass stocks and its distribution among ecosystem compartments

Table 1.1 shows an overview of the results of the stepwise regression models for biomass stocks, carbon flux, and efficiency (see Table S1.5 in Supporting information for a more detailed description). Stand age, MAP, and the length of the warm period were the most correlated predictors with the spatial variability and distribution of the different compartments of biomass.

LAI and foliar biomass were positively correlated with indicators for water availability. Both were, respectively, 1.3 and 3 times higher in needleleaved than in broadleaved forests. Also, stand age and nitrogen revealed a significantly positive relation with LAI, whereas temperature during the warm period had a negative influence. Despite presenting a negative trend from boreal broadleaved to tropical broadleaved forests (Table S1.2, Supporting information), SLA was not significantly correlated with any of the predictor variables used in this study.

Woody, aboveground, belowground, and total biomasses increased with the logarithm of stand age. Precipitation and the length of the warm period were also positively correlated with these variables of biomass, whereas mean temperature of the warm period negatively influenced total and belowground biomass; the latter was also about 1.5 times higher in needleleaved than in broadleaved forests (Table 1.1).

Younger evergreen forests showed higher proportion of leaf biomass than other forest types, particularly when seasonality of precipitation, nitrogen deposition, and water deficits were low (Table 1.1). The percentage of woody biomass increased with AET and the age of the forest. Interestingly, the percentage of belowground biomass was negatively correlated with precipitation of the warm period, the opposite of the other variables of biomass. In addition, longer warm periods, higher age, and the evergreen leaf habit increased the percentage of biomass assigned to roots.



	AET	PET	WD	Temp	Prec	ThA	PS	LWP	Age	ND	L Habit	L Type	MNG
LAI	🟥 w			🟦 w	🟥				🟥	🟥		N > B	
SLA													
FB			🟦									N > B	
WB					🟥			🟥	🟥				
AB					🟥			🟥	🟥				
BB				🟦 w	🟥			🟥	🟥			N > B	
TB				🟦 w	🟥			🟥	🟥				
%FB			🟦 w				🟦		🟦	🟦	E > D		
%WB	🟥	🟦							🟥				
%BB					🟦 w			🟥	🟥		E > D		
GPP	🟥 w				🟥 w	🟦			🟥				
TBP			🟦					🟥	🟦				
ABP			🟦 w					🟥		🟥			
FNPP								🟥					
WNPP			🟦 w							🟥			
BBP						🟦			🟦				
Re			🟦	🟥	🟥 w								
NEP										🟥			M > UM M > D
ABP%										🟥			
BBP%					🟦 w				🟦				
FNPP%						🟥		🟥					
WNPP%										🟥			
CUEe									🟥				M > UM
BPE							🟦		🟦				
LUE				🟦 w									
WUE		🟦	🟥 w			🟦			🟥				

β	<-1	>-1	>-0.8	>-0.6	>-0.4	>-0.2	0	<0.2	<0.4	<0.6	<0.8	<1	>1

Table 1.1: The influence of climatic variables, stand age, leaf habit, leaf type, and management (MNG) on the biomass, flux, and efficiency variables of the forests. Squares indicate β weights of the stepwise regression models using a color gradient where red indicates positive, blue indicates negative, and white indicates no correlation. In the table, "w" indicates that the relationship was with the variable calculated for the warm period. Otherwise, the relationship was with the annual variable. **Abbreviations:** actual and potential evapotranspiration (AET and PET), water deficit (WD), mean temperature (Temp), total precipitation sum

(Prec), annual thermal amplitude (ThA), precipitation seasonality (PS), length of the warm period (LWP), nitrogen deposition (ND). Annual and warm-period variables of AET, PET, WD, Temp, and Prec are represented in single columns to compact the table. Factor codes: leaf habit (E: evergreen, D: deciduous); leaf type (N: needleleaved, B: broadleaved); management (M: managed, UM: unmanaged, D: disturbed).

3.2.2. Correlations with carbon fluxes

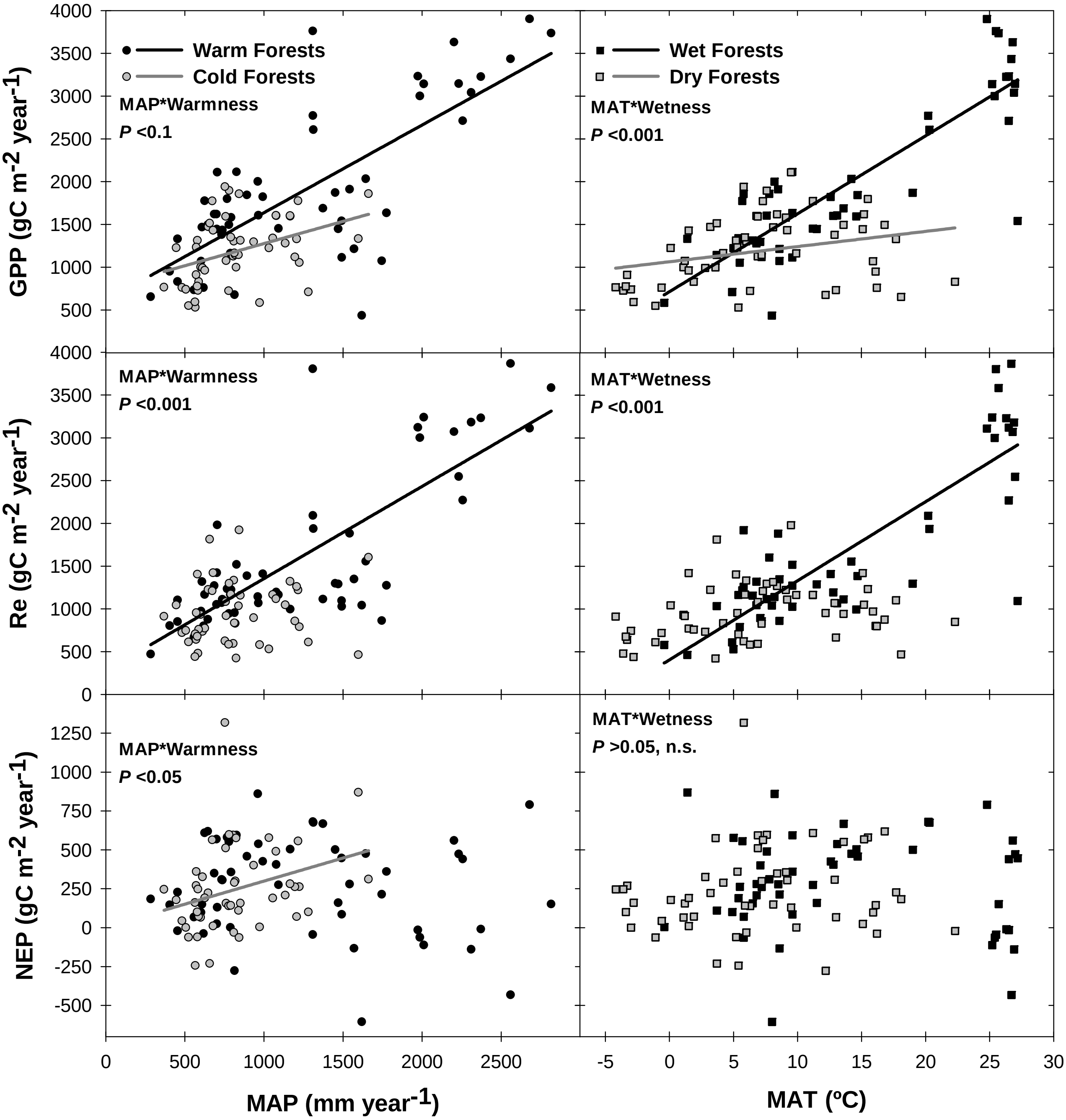
On a global scale, most of the fluxes (except NEP) were strongly influenced by climate variables (Table 1.1 and Table S1.5b, Supporting information). GPP was positively correlated with water availability (precipitation and AET) during the warm period and also by stand age but was negatively correlated with annual thermal amplitude. Re was correlated by the variables of water availability and MAT (Table 1.1), with warm and wet forests presenting the highest rates of respiration. NEP, on the other hand, did not directly respond to climatic variables but was significantly correlated with nitrogen deposition and management. We tested whether this effect was not more prominent in North America and Europe than in the rest of the world, but the models indicated that the relationship did not vary among continents. The rates of NEP were about twice as high in managed as compared to unmanaged forests and 2.6 times higher in managed than in disturbed forests.

Comparison of warm versus cold forests and wet versus dry forests (Figure 1.4), however, revealed that the relationships of GPP, Re, and NEP with MAP depended on warmness, whereas the relationships of GPP, Re, and NEP with MAT depended on wetness (MAP*Warmness: $P < 0.0001$; MAT*Wetness: $P < 0.001$; Figure 1.4), and these relationships differed for each carbon flux. Warm forests revealed a significantly positive relation for both GPP and Re with MAP, both presenting a slope close to unity, while NEP was not correlated at all. Cold forests, instead, showed a lower slope for GPP versus MAP (single linear regression: 0.52; $P < 0.01$), Re was not significantly correlated to MAP (0.17; $P = 0.34$), and NEP revealed a significantly positive relationship with MAP (0.30; $P = 0.03$). Similarly, wet forests showed a significant positive relationship of GPP and Re with MAT, whereas NEP was not significantly related to MAT. Dry forests showed a positive relation between GPP and MAT albeit with a lower slope than the wet forests, whereas Re showed no significant relationship with MAT (Figure 1.4). However, NEP was not significantly related to MAT. Even when excluding the tropical forests (which strongly influenced our regressions, see Figure 1.4) from these analyses, similar patterns were observed (MAP*Warmness: $P < 0.05$; MAT*Wetness: $P < 0.05$ respectively) and remained significant despite presenting less evident changes in the slopes. With the exclusion of tropical forests, however, GPP presented a higher slope with MAP in cold forests than in warm forests, and NEP presented a significant relationship with MAT in wet forests whereas NEP was not longer related with MAP in cold forests.

The path analysis relating AET, MAT and nitrogen deposition with GPP, Re and NEP (Figure 1.5, Table 1.2) showed that AET and MAT only affected Re indirectly through their positive relationship with GPP. Also, nitrogen deposition presented a negative correlation with Re which, in turn, resulted in a positive and significant correlation with NEP (Table 1.2) just as the stepwise regression analysis revealed (Table 1.1). The relationship between GPP and Re was very tight (Figure 1.5) and the calculated total effects for NEP revealed a greater sensitivity to Re (standardized coefficient for Re = -2.55 ± 0.05) than to GPP (0.26 ± 0.11) (Table 1.2).

High TBPs were correlated with low water deficit and long warm periods but decreased with increasing stand age (Table 1.1). Forests with long warm periods produced more foliage and aboveground biomass compared to forests with shorter warm periods. Also, aboveground biomass and wood biomass production were higher in forests presenting low water deficit and high nitrogen deposition compared to forests with high water deficit and low nitrogen deposition (Table 1.1).

Figure 1.4: Scatter plots showing how mean annual precipitation (MAP) and mean annual temperature (MAT) are correlated with GPP, Re, and NEP, depending on the climatic characteristics of the stands (Wet: MAP >827 mm year^{-1}; Warm: MAT >8 °C). Only significant slopes (*P*<0.05) have been drawn. The levels of significance of the interaction MAP*MAT are also indicated.



Similarly, forests receiving higher nitrogen deposition assigned higher percentage of carbon to wood and aboveground biomass (Table 1.1). The percentage of carbon assigned to roots, on the other hand, was negatively correlated with precipitation during the warm period (as with the percentage of belowground biomass, mentioned in section 3.2.1) and by stand age. Finally, the percentage assigned to foliar NPP was positively correlated with annual thermal amplitude and the length of the warm period.

Forests with higher LAI and aboveground biomasses also had higher rates of GPP (LAI: $R^2 = 0.14$, $P = 0.001$; aboveground biomass: $R^2 = 0.26$; $P < 0.001$). Also, Re was positively correlated with aboveground biomass ($R^2 = 0.23$; $P < 0.001$), and biomass production was positively correlated with woody biomass ($R^2 = 0.62$; $P = 0.004$). Other relationships emerged but were not significant after correction by Bonferroni procedures ($\alpha = 0.005$).

3.2.3. Correlations with forest resource-use efficiency

Stepwise regressions showed that CUEe and WUE were positively correlated with stand age, while BPE was negatively correlated with stand age (Table 1.1, Table S1.5c, Supporting information). In addition, CUEe was affected by management in the same way as NEP (i.e. CUEe was higher in managed than in unmanaged forests), and the seasonality of precipitation was negatively correlated with BPE. LUE and WUE responded differently to temperature: lower values of WUE were detected in forests with high annual thermal amplitudes (which could also indicate a negative effect of temperature: see Table S1.1, Supporting information), whereas LUE decreased with the temperature of the warm period (although very little variance was explained by the model; Table 1.1 and Table S1.5c, Supporting information). Also, WUE was negatively correlated with PET.

4. Discussion

The relationships found in our global analyses mostly agreed with previous meta-analyses or established ecophysiological principles. However, our analyses also uncovered novel global trends that deserve thoughtful discussion. We must also point out that, unfortunately, fertility could not be directly assessed in this paper and therefore, some relationships reported here might change according to the nutrient status of the forests.

4.1. Forest functioning, characteristics, and endogenous drivers

4.1.1. Biomass stocks and carbon fluxes

We have confirmed that the largest stocks of biomass are found in temperate and tropical forests (Figure 1.1) (Keith et al., 2009). These forests also have the highest rates of biomass production and carbon uptake (Figures 1.2 and 1.3a). In contrast, boreal and especially Mediterranean forests have the lowest stand biomasses. Because boreal forests are strongly limited by temperature and Mediterranean forests by water availability (Kramer *et al.*, 2000), the low biomass stocks of these forests may be a consequence of their slow growth or conservative strategies imposed by strong constraints to growth (Ackerly & Stuart, 2009). Our results agree with this hypothesis: indicators of water availability (MAP, AET, WD) and length of the warm period were the most influential climatic variables on forest structure and functioning (Table 1.1).

Logically, stand age was paramount in controlling woody, aboveground, and belowground biomasses, but its influence on foliage was negligible, suggesting the existence of a threshold to foliar biomass reached at young ages (Ryan et al. 1997; Chen et al. 2007, Table 1.1, Table S1.5a, Supporting information). This positive relationship of age with woody and root biomasses and the lack of a relationship with foliar biomass is surely the cause of the evident negative effect age has on the percentage of foliar biomass. Despite the positive relationship of age with root biomass, root NPP presented a negative response to aging. This result may indicate that the older the forest, the lower the resources required investing in roots, because the necessity to achieve a robust root system (strong anchor structure and large provisioning area) might be fulfilled by the progressive accumulation of root biomass. Furthermore, changes in the ratio fine-to-coarse roots to the belowground stock might change the belowground productivity as fine roots have different turnover rates than coarse roots.

The growth of some tropical forests is limited by solar radiation (Nemani *et al.*, 2003). According to the functional equilibrium hypothesis (Poorter et al., 2012), the limitation of light may account for the high percentage, amount and production of foliar biomass in tropical broadleaved forests (Figure 1.1, Figure 1.2a, Table S1.2 and Table S1.3 in Supporting information). Poorter et al. (2012), however, suggested that this limitation of light should also lead to high values of SLA more than to high values of the percentage of foliar biomass at the plant level, but this is exactly opposite to our results at the ecosystem level (Table S1.2, Supporting information). This issue requires further examination as other evolutionary issues, such as herbivory, could be involved in SLA variation. On the other hand, needleleaved forests showed rather low values of SLA and higher amounts and percentages of foliar biomass, although GPP did not differ between leaf types within biomes. This observation agrees with the idea that needles photosynthesize less efficiently than broadleaves (Lusk *et al.*, 2003).

The distribution of biomass among compartments is quite similar among forest biomes, with the exception of the Mediterranean forests, where roots represent ca. 50% of the total biomass (Pausas, 1999). This exception is the result of evolutionary adaptations to withstand summer droughts or repeated fires by the accumulation of nonstructural carbohydrates in the lignotuber (Ackerly & Stuart, 2009). Our results support this hypothesis; we found that the percentage of belowground biomass decreased with increasing amounts of precipitation during the warm period (Table 1.1 and Table S1.5a, Supporting information). Belowground biomass (or fraction), however, did not correlate with belowground biomass production (or carbon partitioning to roots), which may indicate that this higher amount of root biomass is more likely the result of a progressive accumulation than of higher carbon partitioning into roots. Because estimates of single-site root biomass stocks and production are very uncertain (as reported by Robinson [2004], for example), studies like ours that synthesise data represent a useful approach for acquiring reliable measures of belowground biomass (assuming that single-site uncertainty is random). However, forest biomes with few available data (see Figure 1.1) may still require caution and further evaluation.

The positive relationship between GPP and stand age indicates that assimilation in forests is not only driven by climatic characteristics, as some models assume, and calls for a renewed attention from the forest carbon modeling community to the relationships between age and canopy or foliar characteristics. Despite the higher GPP of tropical forests than temperate forests, NEP is higher in temperate than in tropical forests. In fact, our data indicate that although tropical forests are most likely to be sinks of carbon (Stephens *et al.*, 2007; Lewis *et al.*, 2009), the average NEP in our study is not statistically significantly different from zero (Table S1.3, Supporting information) (Pan *et al.*, 2011). Accordingly, tropical forests showed the lowest CUEe and BPE ratios of all forests (Figures 1.2 and 1.3b), indicating that they are the least efficient in the use of GPP, possibly because of the high temperatures and water availabilities that enhance both autotrophic and heterotrophic respiration more than rates of GPP (Figure 1.4, Chambers et al., 2004; Kato and Tang, 2008) and/or because of the low nutrient availability that could enhance the production of root exudates or other unaccounted for components of NPP (Goulden *et al.*, 2011; Vicca *et al.*, 2012). On the other hand, our results indicated that spatial variability in NEP was more sensitive to changes in Re than in GPP (Table 1.2) which agrees with results from previous studies (Valentini *et al.*, 2000).

The decrease of BPE with age was one of the foundations used by Goulden et al. (2011) to suggest that in boreal forests decreasing nutrient availability with increasing age could enhance carbon partitioning into the fine-root fraction. Our results support this negative relationship of stand age with BPE, but stand age also presented a negative relationship with the fraction of GPP partitioned to root (fine + coarse) biomass and a positive relationship with the amount and percentage of belowground biomass (Table 1.1). These results do not necessarily contradict the hypothesis presented by Goulden et al. (2011), because our data did not differentiate from the fine-root to the coarse-root fraction. An overall decrease in the production of belowground biomass could also be possible if an increase in the fine-root fraction is compensated for by a decrease in the production of coarse-root biomass. Also, Goulden et al. (2011) reported a negative effect of age on the ratio of NEP to TBP, indicating that old stands approach a steady state. We obtained a similar pattern for CUEe (as the ratio of NEP to GPP), as efficiency of carbon sequestration increased with age and tended to reach a steady state with positive values of CUEe (because of a logarithmic relationship;

Table 1.1 and Table S1.5c, Supporting information). However, the predicted confidence intervals of CUEe did not differ from zero.

4.1.2. Light and water-use efficiency

The similar values of LUE and WUE found across biomes (Table S1.4, Supporting information) suggest a convergence in the average of these resource-use efficiencies of forest ecosystems on a global scale (Goetz and Prince, 1999; Huxman et al., 2004). On the other hand, the considerable variability observed within biomes and the lack of LUE data for tropical forests in particular, may obscure existing trends.

Binkley *et al.* (2004) stated that, at the ecosystemic level, an increase in efficiency modulated by an increase in a resource use could be due to an increased "*return on investment*" (e.g. a canopy with good hydric conditions would photosynthesise more carbon than another suffering stomatal closure for the same investment in canopy). Old forests had higher WUEs, possibly due to i) the positive relationship of stand age with LAI and GPP (given that we calculated $WUE=GPP{\cdot}AET^{-1}$; Table 1.1, Magnani *et al.*, 2007) and ii) an increase in the resources (e.g. water, nutrients) provided by the root system to the canopy as a result of increasing root biomass with age (Table 1.1) (Binkley *et al.*, 2004; Ackerly & Stuart, 2009). In agreement with Binkley's hypothesis, we found a significant relationship between LAI and GPP similar to that reported by other authors (Gower et al., 2001; Law et al., 2002; Kato and Tang, 2008).

4.2. Exogenous drivers of forest functioning and characteristics

4.2.1. The role of climate

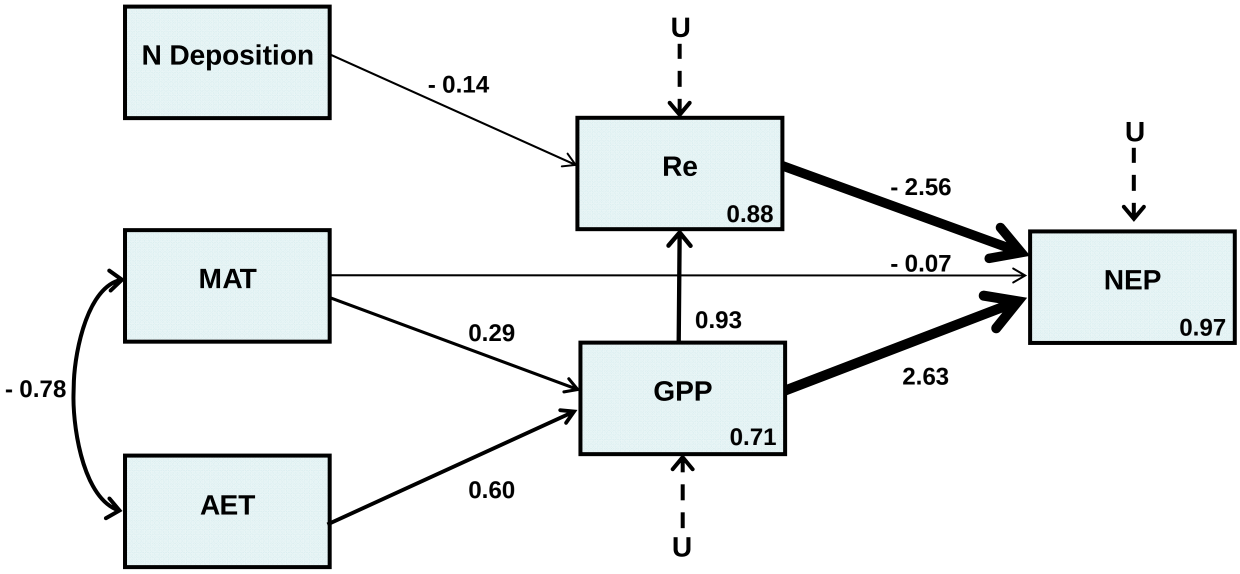
Our analyses confirm that water availability and the length of the warm period (~temperature) are paramount for controlling forest structure and functioning (Luyssaert *et al.*, 2007; Keith *et al.*, 2009). Only SLA, NEP, ABP%, WNPP%, and CUEe (5 of 26 variables studied) were not related to climate variables. Carbon pools and fluxes were generally higher in warm and wet forests (Table 1.1).

We have refined the analysis of Luyssaert *et al.* (2007), distinguishing dry from wet and cold from warm forests, and adding insights into the relationship between NEP and climate. Our results suggest that the interaction between MAT and MAP is of paramount importance for understanding the effect of climate on NEP (Figure 1.4). The relationships of GPP and Re with MAP in warm forests are very similar and prevent a relationship between NEP and MAP, because most of the carbon assimilation is counterbalanced by the ecosystem respiration. In cold forests, however, the effect of MAP on GPP is stronger than the effect of MAP on Re, and consequently NEP is positively influenced by MAP. These relationships suggest that, on a global scale, Re respond differently than GPP to the interaction between MAT and MAP, which is reflected by the correlation between NEP and MAP only in cold forests, where part of the production (GPP) is not respired due to limitations of temperature.

On the other hand, when looking for indirect relationships between climate and carbon fluxes, we found that Re does not present direct relationships neither with MAT nor with AET. Instead, the relation of Re with climate goes through the direct relationship with production (GPP, Figure 1.5) as suggested by previous studies (Janssens *et al.*, 2001). We found NEP to be directly correlated with MAT, but the correlation, although significant, was almost meaningless and was not significant when accounting for the total effects (Table 1.2).

The positive influence of water deficit during the warm period on WUE (Table 1.1)(Huxman et al., 2004; Yu et al., 2008; Troch et al., 2009) suggests an adaptive trait to maximise the efficiency of water use in those forests growing under water-limited conditions (Aranda *et al.*, 2007; Ackerly & Stuart, 2009). Our results also indicated that forests with lower thermal amplitudes can use water more efficiently, in terms of gross carbon fixation, than those exposed to wider oscillations in temperature. This relationship may be explained by several hypotheses: i) in evergreen forests, extremely cold episodes can provoke massive degradation of chloroplast, which can take up to eight weeks to recover (Malhi et al., 1999), ii) in warm forests presenting high thermal amplitude, elevated rates of evapotranspiration caused by extremely warm temperatures at the leaf and canopy levels can lead to higher photorespiration and thus lower GPP (Malhi et al., 1999), or iii) because warmer forests tend to have lower thermal amplitudes (Table S1.1, Supporting information), the longer periods of photosynthetic activity in warmer forests may enhance carbon uptake, whereas AET may remain constant on a yearly basis (if AET is limited by precipitation), thereby yielding higher WUEs. Additionally, lower thermal amplitudes also reduce the risk of damage from extremely cold or warm events.

Figure 1.5: Resulting diagrams of the path analysis relating climate (AET and MAT) and nitrogen deposition as exogenous variables with carbon flux variables (GPP, Re and NEP) as endogenous variables. The "U" represents the non-explained variance of the endogenous variables, the arrows show the standardized coefficients of the path (direct effects) and the boxes of the endogenous variables show the variability explained (R^2). All paths shown are significant and models were simplified to achieve the Minimum Adequate Model according to AICc.



On the other hand, WUE was negatively related to PET. In dry forests, higher PET creates larger water-potential gradients between the stomata and the atmosphere, higher rates of evapotranspiration per unit of carbon gained (lower WUE), and hence stomatal closure by leaf water depletion, leading to increased foliar temperature which, in addition, increases photorespiration (Malhi et al., 1999; Yu et al., 2008). Also, high levels of PET lead to high rates of evapotranspiration in forests without water restrictions (e.g. humid tropical forests), which could also reduce WUE. Similarly, forests with warmer growing seasons may have lower values of LUE (Table 1.1) due to enhanced photorespiration produced at higher temperatures or due to a higher probability of stomatal closure caused by the high evaporative demand, thereby reducing photosynthesis per unit of APAR.

Total Effects	**N dep**	**AET**	**MAT**	**GPP**	**Re**
GPP		**1.60 ± 0.23**	**27.44 ± 8.08**		
Re	**-19.06 ± 4.58**	**1.46 ± 0.22**	**25.01 ± 7.42**	**0.91 ± 0.03**	
NEP	**18.44 ± 4.44**	0.16 ± 0.31	0.22 ± 10.74	**0.09 ± 0.04**	**-0.96 ± 0.02**
Standardized total effects					
GPP		**0.60 ± 0.09**	**0.29 ± 0.09**		
Re	**-0.14 ± 0.05**	**0.55 ± 0.08**	**0.27 ± 0.08**	**0.93 ± 0.03**	
NEP	**0.37 ± 0.14**	0.16 ± 0.31	0.01 ± 0.30	**0.26 ± 0.11**	**-2.55 ± 0.05**

Table 1.2: Total effects (mean ± SE) resulting from the path analysis shown in Figure 1.5. Bold coefficients were significant at the 0.05 level.

4.2.2. The role of management

Previous studies (Shan et al., 2001 and references therein) have suggested that management can alter the patterns of carbon allocation through an increase in the available resources due to a relaxation of competition. Shan et al. (2001) reported increases in early volume growth and in the ratio of shoots to roots after elimination of the understory. Despite the potential effect that management can have in forest ecosystems, our analyses confer little importance to it. We have not detected changes in the patterns of carbon allocation, rates of production, or biomass stocks as a result of management (Table 1.1). This is maybe partially due to the fact that we used a global dataset of forests for which each variable had a wide range of variation. Thus, changes in these variables may depend more on the characteristics of the climate than on management. In the present study, only NEP and CUEe were directly related to management. Reducing biomass stocks and lowering competition for light, water, and nutrients seem a key for increasing NEP and CUEe, although management had no significant effect on carbon uptake or ecosystem respiration.

4.2.3. The role of nitrogen deposition

Nitrogen deposition also had a relevant influence on the functioning of forest ecosystems, as previously reported by other authors (see De Vries et al., 2009; Janssens et al., 2010; Luyssaert et al., 2010; Magnani et al., 2007). For example, the increased biomass production and carbon allocation to wood with increased nitrogen deposition (Table 1.1) agrees with the paradigm that partitioning to aboveground production increases with increasing belowground resources (Litton *et al.*, 2007). Also, higher nitrogen availability allows to maintain higher nitrogen concentrations and consequently higher photosynthetic capacity per unit of leaf area (Field *et al.*, 1983). We also found nitrogen deposition to positively influence LAI and NEP (Table 1.1). This influence may be related to an increase in the availability of nitrogen for plants, rendering nitrogen a less limiting factor to growth. Also, the negative influence of nitrogen deposition over the percentage of foliar biomass (Table 1.1) may indicate a shift towards more productive leaves when nitrogen is available. Regarding the effect on NEP, our path analysis revealed that the effect in NEP is, in fact, mediated by the effect of nitrogen deposition in Re (Figure 1.5 and Table 1.2). In this sense, nitrogen deposition has also been suggested to reduce heterotrophic respiration (Janssens et al., 2010) by means of: i) changes in the saprotrophic community towards one more efficient in the use of carbon, which requires higher nitrogen availability, and ii) abiotic mechanisms of soil organic matter stabilisation, producing nitrogenous compounds that reduce the decomposability of soil organic matter. The maximum value of nitrogen deposition found in our forests was 27.3 kg ha^{-1} $year^{-1}$; higher amounts of nitrogen deposition may have opposite consequences in forest ecosystems (e.g. very high nitrogen deposition could cause negative impacts on carbon sequestration, De Vries et al., 2009).

Hence, this study provides an updated description of the structure, functioning, production, and resource-use efficiency of forests located in the main forest biomes of our planet. We also indicate the main controls of these properties, highlighting the paramount role of water availability, temperature and stand age. The results show a global positive synergic effect of MAP and MAT on forest production, growth and carbon accumulation. Although climate has globally affects BPE, LUE, and WUE, mean efficiencies differed very little among biomes, suggesting a global convergence of resource-use efficiencies across ecosystems.

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Chapter 2

Nutrient availability as the key regulator of global forest carbon balance

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Abstract

Forests strongly affect climate through the exchange of large amounts of atmospheric CO_2 (Dixon *et al.*, 1994). The main drivers of spatial variability in net ecosystem production (NEP) on a global scale are, however, poorly known. Since increasing nutrient availability increases the production of biomass per unit of photosynthesis (Vicca *et al.*, 2012) and reduces heterotrophic (Janssens *et al.*, 2010) respiration in forests, we expected nutrients to determine carbon sequestration in forests. Our synthesis study of 92 forests in different climate zones revealed that nutrient availability indeed plays a crucial role in determining NEP and ecosystem carbon-use efficiency [CUEe, i.e. the ratio of NEP to gross primary production (GPP)]. Forests with high GPP exhibited high NEP only in nutrient-rich forests (CUEe = 33 ± 4%; mean ± SE). In nutrient-poor forests, a much larger proportion of GPP was released through ecosystem respiration, resulting in lower CUEe (6 ± 4%). Our finding that nutrient availability exerts a stronger control on NEP than on carbon input (GPP) conflicts with assumptions of nearly all global coupled carbon cycle-climate models, which assume that carbon inputs through photosynthesis drive biomass production and carbon sequestration. An improved global understanding of nutrient availability would therefore greatly improve carbon cycle modeling and should become a critical focus for future research.

1. Main text

The net ecosystem production (NEP) of an ecosystem represents its C balance at daily to decadal scales. Despite considerable study, the main drivers of NEP are still unclear. Climate (Valentini *et al.*, 2000; Kato & Tang, 2008), climatic trends (Piao *et al.*, 2009), nitrogen deposition (De Vries *et al.*, 2009; Fernández-Martínez *et al.*, 2014), disturbance and management (Luyssaert *et al.*, 2007; Fernández-Martínez *et al.*, 2014) have been suggested to influence NEP. These studies, however, were either unable to explain a substantial percentage of the spatial variability in NEP or collected data in a restricted subset of climatic space, indicating that it is not yet known what factor(s) most strongly govern NEP, one of the critical pathways by which terrestrial ecosystems feedback to climate.

At the ecosystem scale, nitrogen deposition has been suggested to enhance the NEP of forests (De Vries *et al.*, 2009; Janssens *et al.*, 2010). Nutrient availability is indeed a key variable explaining patterns of carbon allocation in forests; nutrient-rich forests exhibit higher biomass production (BP), biomass production efficiency (BPE, defined as BP-to-GPP ratio) and shoot-to-root biomass production ratio (Vicca *et al.*, 2012). By converting a larger fraction of GPP to woody biomass and thereby increasing the residence time of the assimilated carbon (C), forests growing on more fertile soils can be expected to exhibit higher NEP. Carbon-use efficiency at the ecosystem level (CUEe), defined as NEP of an ecosystem per unit of GPP, measures the proficiency of an ecosystem to store C absorbed from the atmosphere. We thus hypothesize that both NEP and CUEe increase with increasing nutrient availability in forest ecosystems.

To test this hypothesis, we updated and analyzed a global forest data set of mean annual carbon flux [GPP, ecosystem respiration (Re) and NEP], stand biomass, stand age and information on management. The resulting data set of 92 forests included scattered data from 1990 to 2010 from boreal, temperate, Mediterranean and tropical forests (Luyssaert *et al.*, 2007) (Figure S2.1). We added all published information on the nutrient status of these forests and we classified them as forests with high nutrient availability (without apparent nutrient limitation) and low nutrient availability (apparently strongly nutrient-limited, sensu Vicca et al. (Vicca *et al.*, 2012), considering a holistic combination of availability of nutrients and soil characteristics). We based the nutrient availability classification on a multivariate factor analysis containing information about soil type, soil and foliar nutrient concentrations (N, P), soil pH, soil C:N ratio, nitrogen deposition and mineralization, history of the stand, specific reports of nutrient availability and an assessment by the principal investigator of the site (Table S2.1). This analysis clearly separated nutrient-rich from nutrient-poor forests (Figure S2.2). We also established a medium category that was used for additional testing; it contained forests with information indicating moderate availability of nutrients or with little information about their nutrient status. Mean annual temperature and precipitation (MAT, MAP) from the WorldClim database(Hijmans *et al.*, 2005) and water deficit (WD) derived from MODIS evapotranspiration time series (MOD15A2 product) were used as climatic predictors. We then used generalized linear models to disentangle the effects of climate, management and stand age from those of nutrient availability on NEP and CUEe (see Methods for details on datasets and methodology).

NEP in nutrient-rich forests averaged 33 ± 4% (mean ± SE) of GPP, whereas nutrient-poor forests only accumulated 6 ± 4% of the photosynthesized carbon (CUEe in Figure 2.1, difference = 27 ± 7% , ANOVA $P < 0.001$). Only nutrient-rich forests showed a clear positive relationship between GPP and NEP (Figure 2.1). In contrast, nutrient-poor forests channelled a larger proportion of GPP into Re (Figure 2.2), with NEP being almost independent of GPP. Higher nutrient availability thus appears to channel C fixed by GPP toward storage in biomass and soils, rather than being respired back to the atmosphere.

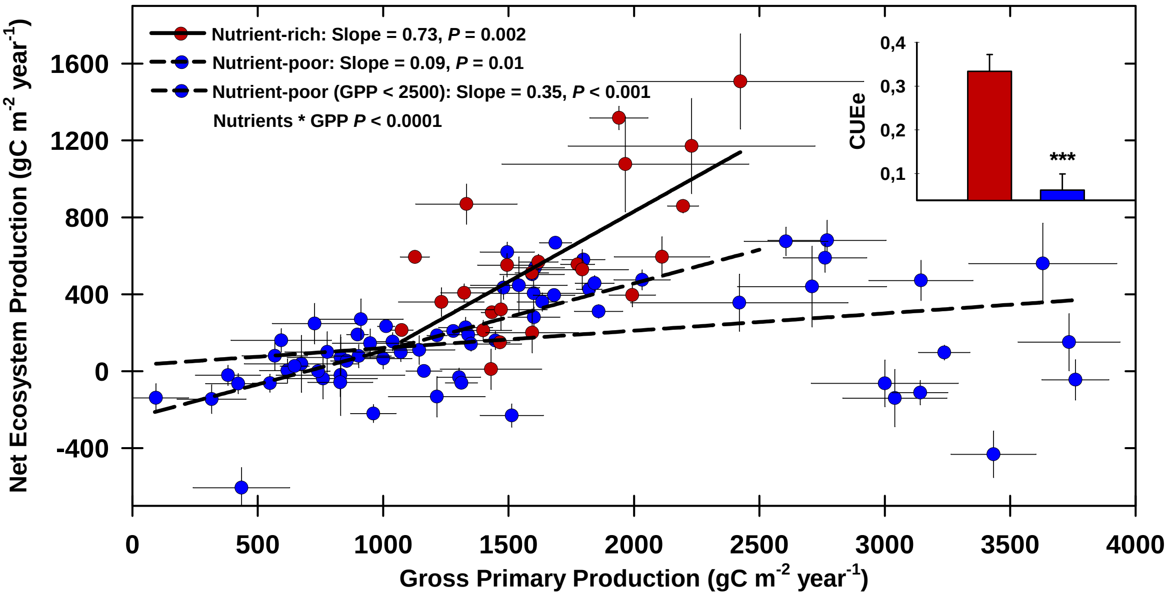

Figure 2.1. Only nutrient-rich forests substantially increase carbon sequestration with increasing carbon uptake. The bar chart inside the main graph shows that CUEe (NEP to GPP ratio) in nutrient-rich forests is more than five times higher than in nutrient-poor forests. We also present results for forests with GPP < 2500 gC m^{-2} $year^{-1}$, because values of GPP > 2500 gC m^{-2} $year^{-1}$ were only available for nutrient-poor forests. When considering only forests with GPP < 2500 gC m^{-2} $year^{-1}$, the Nutrients*GPP (where Nutrients = nutrient availability) interaction was significant at the 0.006 level.

A common protocol in eddy covariance CO_2 flux studies is to estimate GPP by adding Re (e.g. extrapolated from nocturnal measurements) to the measured net ecosystem exchange (NEE, a proxy for short-term NEP). In this protocol any error in Re would therefore be directly propagated into a biased estimation of GPP, potentially imposing a spurious correlation between GPP and Re (Reichstein *et al.*, 2005; Vickers *et al.*, 2009). This correlation, however, in addition to being irrelevant on an annual scale (Lasslop *et al.*, 2010), was present in nutrient-poor forests but not in nutrient-rich forests (Figure 2.2). The correlation between GPP and Re observed across nutrient-poor forests is thus unlikely an artefact from the processing of eddy-covariance data for separating these gross fluxes. We instead hypothesize that the positive relationship between Re and GPP only in nutrient-poor forests is due to different patterns of ecosystem functioning in nutrient-poor versus nutrient-rich forests.

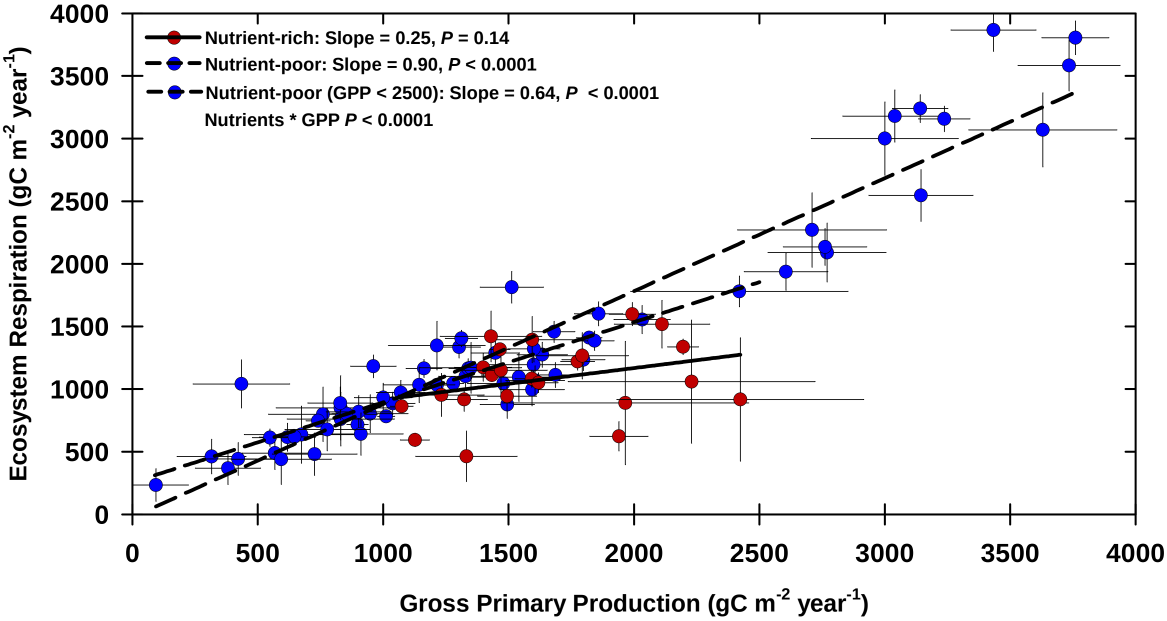

Figure 2.2. The coupling between Re and GPP is weak in nutrient-rich forests and very strong in nutrient-poor forests. Nutrient-rich forests decouple Re from GPP, resulting in an increase in carbon accumulation with increasing GPP. When considering only forests with GPP < 2500 gC m^{-2} $year^{-1}$, the Nutrients*GPP (where Nutrients = nutrient availability) interaction is significant at the 0.005 level. Error bars indicate the uncertainty of the estimate on both the x- and y-axes (SE).

Our statistical analyses using generalized linear models, including GPP, nutrient availability and stand age, explained 74, 93 and 43% of the variance in NEP, Re (Table 2.1) and CUEe across sites, respectively (Table S2.2). Nutrient availability alone explained 19% of the variance in NEP. When summed with its interactions with GPP (15%) and age (1%), nutrient availability accounted for 35% of the variance in NEP. GPP alone explained 18% of the cross-site variability in NEP. When additional interactions with nutrient availability and age (9%) were included, GPP explained 42% of the variability in NEP. The relationship between NEP and stand age, however, was only significant when GPP was previously included in the models, which emphasises the smaller effect of stand age on NEP as compared to GPP (Figures S2.3 and S2.4). Finally, MAT was positively correlated with NEP and explained 9% of its variance. In contrast to NEP, GPP alone explained 64% of the variance in Re, with nutrient availability and its interactions explaining 9% and age and its interactions explaining only 5%. For CUEe, nutrient availability explained 12%, and GPP 14% of the variance in CUEe. Stand age also played an important role, interacting with GPP (reducing the positive effect of GPP on CUEe as forests matured) and explaining 17% of the variance in CUEe.

The relative contribution of explanatory variables thus differed among the NEP, Re and CUEe models, but the key and robust result is that nutrient status was a key factor for NEP and CUEe (Figure 2.3, Table 2.1 and Table S2.2), despite the use of nutrient status as a binary variable (high vs. low nutrient availability). Other possible predictors such as management and climate (MAP and WD), were not selected to enter in the general model by the stepwise model selection procedure, i.e., they did not significantly affect neither NEP nor Re (Table 2.1). Model-averaging techniques (see Supplementary Information) also indicated little importance of climate or management on NEP and Re. In contrast to NEP and Re, GPP was clearly climatically driven, being positively correlated with MAT and negatively correlated with WD, which accounted for 65% and 10%, respectively, of the variance in GPP.

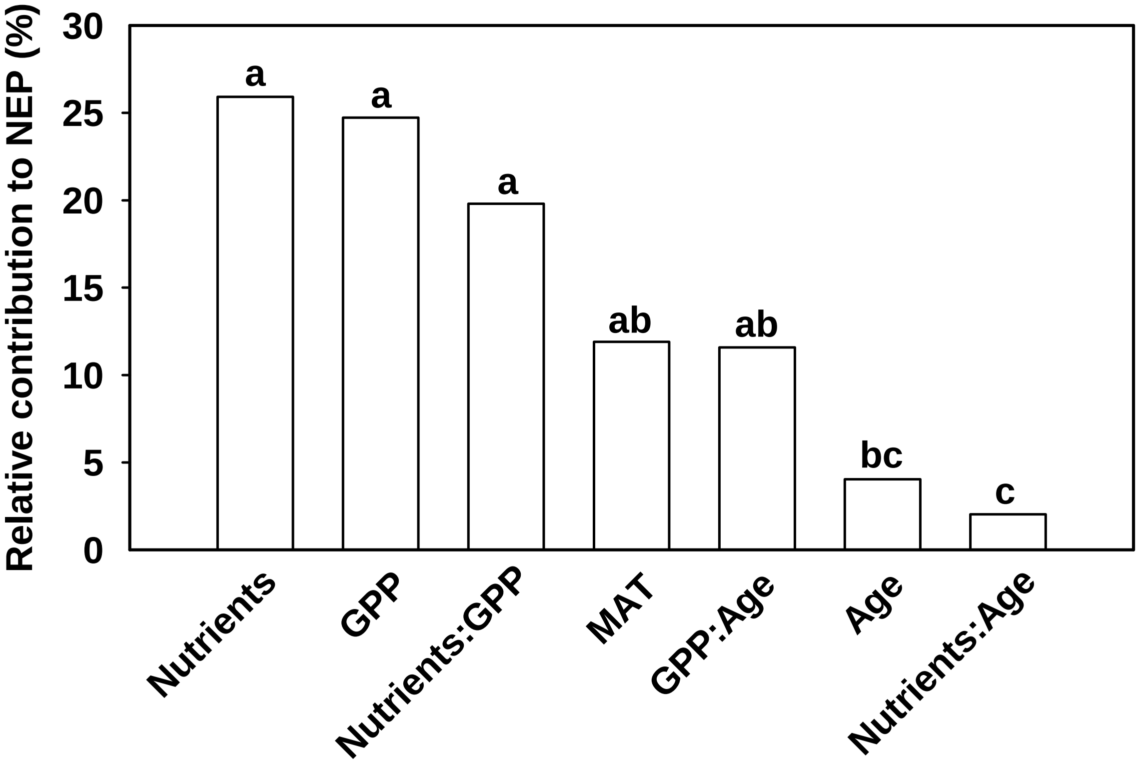

Figure 2.3. Relative contribution of predictor variables in the model explaining variability in NEP. Letters indicate significant differences according to the bootstrapped confidence intervals computed for the differences among variables [relaimpo R package (23)]. Nutrients = nutrient availability. All variables and interactions shown were statistically significant ($P < 0.05$).

The significant positive effect of nutrient availability on NEP proved to be robust in weighted models (Figure S2.5) and when controlling for effects of potentially confounding factors, for example: i) when using only data derived from eddy covariance measurements (Table 2.1), ii) when excluding forests with GPPs > 2500 gC m^{-2} $year^{-1}$ (i.e. mostly tropical forests) from the analyses (no nutrient-rich forests were available for comparison at GPP higher than this threshold, Figures 2.1 and 2.2), iii) when using only managed forests (Figures S2.6 and S2.7), iv) when using an alternative classification of nutrient status to analyse sensitivity to possible classification errors (Tables 2.1 and Table S2.2) and v) when using the first factor of the factor analysis for nutrient classification as a nutrient richness covariate (Table 2.1, *nutrient richness factor*). Furthermore, when including the moderate nutrient availability forests, this group showed an intermediate behaviour between the nutrient-rich and the nutrient-poor forests (Figure S2.8). On the other hand, when nutrient status was excluded from the analyses, management played the role of nutrients in our models, albeit the models explained less of the variance than did the models containing nutrient availability (Table 2.1), and the second-order Akaike information criterion (AICc) increased considerably (by 18.6 and 17.2 points for NEP and Re, respectively). These results were expected because managed forests are mostly nutrient-rich forests (Figure S2.7) for the generation of profits from fertile lands.

The positive effect of nutrient availability on a more efficient use of photosynthates and a larger sequestration of carbon at the ecosystem level is likely not driven by a single mechanism or a single compartment of the ecosystem but rather by a combination of autotrophic and heterotrophic processes. Autotrophic processes are mainly related to different patterns of carbon allocation in nutrient-rich and nutrient-poor forests (Litton *et al.*, 2007; Vicca *et al.*, 2012), whereas mechanisms related to heterotrophic processes involve primarily changes in substrate quality and the composition of the community of decomposers (mainly fungal and bacterial) (Janssens *et al.*, 2010; Manzoni *et al.*, 2012).

For the autotrophic compartment, we detected two differences in the distribution of biomass across different organs between the different nutrient classes, despite also considering other factors such as climate and management. 1) Although only marginally significant, the ratio of fine-root biomass to total biomass was almost three times higher in

Table 2.1. Summary of the percentage of variance explained by the significant variables of the models relating NEP and Re with GPP, nutrient availability (NA), management (MNG) and stand age and their second-order interactions. The β coefficients of the models are shown in brackets. For NA, MNG or their interactions with covariates, the β coefficients of the factors and the interactions indicate differences from the reference level (e.g. the slope of nutrient-rich forests of the general model is 1.8, and the slope, β, of the nutrient-poor forests is 1.8 – 1.9 = -0.1). The model "Nutrient richness factor" shows the model including the factors used in the nutrient classification (see Methods, *information on nutrient availability*, and Figure S2.2) as a nutrient richness covariate instead of the binary variable nutrient availability. For this model, NA indicates the effect of the first factor extracted.

Models	GPP	NA	GPP:NA	MAT	GPP:Age	Age	Age:NA	MNG	WD	GPP:MNG	Model R^2 (%)
General											
NEP (Figure 2.1)	18 (1.8)	19 (1.3)	15 (-1.9)	9 (0.5)	9 (-1.0)	3 (1.1)	1 (-0.4)				74
Re (Figure 2.2)	64 (0.1)	3 (-0.7)	5 (1.1)	16 (-0.2)	3 (0.5)	1 (-0.6)	1 (0.2)				93
CUEe	14 (0.9)	12 (-0.3)			17 (-1.2)	0 (1.1)					43
Weighted (Supl. Figure 2.2)											
NEP	20 (1.6)	14 (1.0)	8 (-1.4)	8 (0.4)	6 (-1.0)	2 (1.2)	3 (-0.5)				61
Re	65 (0.3)	2 (-0.5)	3 (0.8)	15 (-0.2)	2 (0.5)	0 (-0.6)	1 (0.3)				88
CUEe	1 (0.5)	16 (-0.1)			9 (-0.8)	3 (0.9)	5 (-0.4)				34
Eddy covariance data only											
NEP	18 (1.5)	11 (0.8)	6 (-1.5)	9 (0.5)				4 (0.6)		11 (-0.8)	59
Re	67 (0.4)	1 (-0.4)	1 (0.7)	19 (-0.2)				1 (-0.2)		2 (0.4)	92
CUEe	12 (0.9)	9 (-0.3)			15 (-1.2)	2 (1.2)					38
Without nutrient availability											
NEP	31 (1.1)							8 (0.6)	5 (0.3)	15 (-1.8)	59
Re	70 (0.6)							2 (-0.3)	11 (-0.2)	4 (0.5)	87
CUEe	15 (0.9)							0 (0.8)		2 (-1.1)	46
GPP < 2500 gC m^{-2} $year^{-1}$											
NEP (Figure 2.1)	44 (1.2)	17 (0.6)	6 (-0.9)						5 (0.2)		72
Re (Figure 2.2)	55 (0.3)	3 (-0.6)	6 (0.8)						10 (-0.2)		74
CUEe	38 (0.8)				7 (-0.8)	1 (0.9)					46

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Models	**GPP**	**NA**	**GPP:NA**	**MAT**	**GPP:Age**	**Age**	**Age:NA**	**MNG**	**WD**	**GPP:MNG**	**Model R^2 (%)**
GPP < 2500 gC m^{-2} $year^{-1}$ weighted (Supl. Figure 2.2)											
NEP	34 (0.9)	11 (0.7)	5 (-0.9)	12 (0.3)							**62**
Re	58 (0.9)	3 (0.2)							11 (-0.2)		**72**
CUEe		15 (-0.2)		19 (0.3)							**34**
Managed Forests (Supl. Figure 2.6)											
NEP	52 (1.1)	14 (0.3)	4 (-0.7)						9 (0.3)		**79**
Re	57 (0.4)	3 (-0.4)	5 (0.7)						17 (-0.3)		**82**
CUEe	37 (0.7)	9 (-0.3)			5 (-0.6)	3 (0.8)					**54**
Alternative classification											
NEP	25 (1.6)	12 (1.2)	11 (-1.5)	11 (0.5)	1 (-1.1)	4 (1.2)	2 (-0.4)				**75**
Re	67 (0.3)	2 (-0.8)	4 (0.9)	13 (-0.3)	4 (0.6)	1 (-0.7)	1 (0.2)				**92**
CUEe	12 (0.6)	7 (-0.3)		6 (0.4)	18 (-1.2)	0 (1.2)					**43**
Nutrient richness factor											
NEP	25 (0.9)	23 (-0.4)	5 (0.8)					5 (0.4)		9 (-0.7)	**67**
Re	79 (0.7)	4 (-0.2)						1 (-0.3)		3 (0.4)	**87**
CUEe	14 (0.8)	10 (0.2)						0 (0.8)		17 (-1.0)	**41**

nutrient-poor forests than in nutrient-rich forests (*P* = 0.06, N = 17; Figure S2.9A), indicating a higher proportional investment of GPP into fine roots for increasing access to nutrients (Shan *et al.*, 2002; Goulden *et al.*, 2011). 2) The leaf area index per unit of fine-root biomass was twice as large in nutrient-rich forests (*P* = 0.013, N = 19; Figure S2.9B), indicating a shift in carbon allocation towards photosynthetic tissues when nutrients are not limiting growth and trees need to invest less in nutrient-acquiring structures. Accordingly, an earlier study, using a subset of our database, pointed out that nutrient-rich forests allocate larger proportions of their photosynthates to wood production compared to nutrient-poor forests at the cost of producing less root biomass (Vicca *et al.*, 2012). These changes in allocation patterns thus suggest enhanced carbon fixation in nutrient-rich forests.

An increase in the production of leaves in nutrient-rich forests, at the expense of producing less fine roots, could decrease the benefit of increasing aboveground allocation in terms of CUEe if that aboveground carbon is not stabilised. On the other hand, although some studies have reported higher root respiration per unit mass at high root nutrient concentrations (Chapin, 1980; Burton *et al.*, 2002), a substantial decrease in root biomass may counterbalance this increase in autotrophic respiration and even reduce it at the ecosystem level (Janssens *et al.*, 2010). In addition, when soil nutrients are poorly available, plants engage in active nutrient transport through the cell to increase nutrient uptake, spending energy for nutrient acquisition and therefore reducing energy available for plant growth(Lee *et al.*, 2007). The net effect of root physiological adjustments to nutrient supply is unclear.

Changes in patterns of photosynthate allocation are also relevant for the heterotrophic compartment. For example, the higher proportion of GPP in nutrient-rich forests partitioned to tissues with long turnover times such as wood (Litton *et al.*, 2007; Vicca *et al.*, 2012) may decrease heterotrophic respiration, because wood is generally composed of rather recalcitrant molecules that decompose slowly (Keith *et al.*, 2009). Furthermore, numerous studies suggest that under high nutrient availability, forests allocate less C to fungal root symbionts (Vicca *et al.*, 2012), and to exudation that stimulates heterotrophic respiration in the rhizosphere (Janssens *et al.*, 2010). Together, these nutrient effects would reduce microbial biomass and respiration, relative to nutrient-poor forests. In addition, communities of microbes and detritivores that consume nutrient-rich organic matter have higher growth efficiencies (less respiration per unit of organic matter decomposed) than do communities that decompose nutrient-poor organic matter (Manzoni *et al.*, 2012; Cotrufo *et al.*, 2013). This difference could reduce heterotrophic respiration in nutrient-rich forests (Janssens *et al.*, 2010; Manzoni *et al.*, 2012) and potentially enhance carbon sequestration and accumulation in nutrient-rich forests.

Our results indicate a key effect of nutrient availability on forest carbon balance and particularly on the capacity of forests to sequester carbon. Only when nutrient availability is high can forests sequester large amounts of carbon. This knowledge is crucial, especially given the human-induced alterations of nutrient availability and stoichiometry in many regions of the planet (Peñuelas *et al.*, 2012, 2013). Earth system models should evolve from considering only the effects of nitrogen on plant growth (Zaehle & Friend, 2010; Zaehle *et al.*, 2010) to considering the interactions of nitrogen as well as other nutrients with the entire carbon cycle (de Vries & Posch, 2011). The relationship between GPP and NEP appears to be strongly controlled by the nutrient status of the forest, which implies that Earth system models will be unable to accurately predict the carbon balance of forest ecosystems without information on both background (pre-industrial) and regional changes in nutrient availability (Piao *et al.*, 2013) resulting from direct human activities (e.g. nitrogen deposition) and from indirect human activities (e.g. climate change and elevated CO_2 altering soil and plant nutrient cycling). Moreover, because GPP and surrogates are widely available from remotely sensed data, the assessment of nutrient status could allow estimation of NEP with remote sensing of GPP and ground based measurements of CUEe. This way, estimates of global terrestrial carbon sequestration could be improved, and guidance for improved management of forest carbon could be provided. Finally, experimental research and environmental monitoring would benefit substantially by considering nutrient availability as carefully as climate.

2. Methods

Sources of data

We used data of mean annual carbon flux from a global forest database (Luyssaert *et al.*, 2007). This data set contains complete measurements of carbon balance and uncertainties of gross primary production (GPP), ecosystem respiration (Re) and net ecosystem production (NEP) of forests around the world. The WorldClim database (Hijmans *et al.*, 2005) (resolution ~ 1km at the equator) and MODIS evapotranspiration time series (MOD15A2 product) provided climatic data [mean annual temperature (MAT) and mean annual precipitation (MAP) from WorldClim and potential and actual evapotranspiration (PET, AET) from MODIS]. The reliability of the data from the WorldClim database was tested with the available observed climatic values from the forests (N=123). Results indicated a strong correlation between observed and WorldClim values for annual temperature and precipitation ($R^2 = 0.96$, $P < 0.001$ and $R^2 = 0.84$, $P < 0.001$ respectively).

All continents were represented in our analyses (Figure S2.1), although most of the forests were located in Europe and North America. Boreal (N = 31) and especially temperate (N = 68) sites outnumbered Mediterranean (N = 14) and tropical (N = 16) sites. 61 forests were coniferous, 57 were broadleaved and 11 were mixed.

Information on nutrient availability

For each forest, we compiled all available information from the published literature (carbon, nitrogen and phosphorus concentrations of soil and/or leaves, soil type, soil texture, soil C:N ratio, soil pH, measures of nutrients, etc.) related to nutrient availability. Then we followed the criteria shown in Table S2.3 to code these variables as three-level factors indicating high, medium or low nutrient availability. Next, we transformed these factors into dummy variables and performed a factor analysis. The first factor extracted explained 14.8% of the variance in the dataset and was related to nutrient-rich dummy variables whereas the second factor explained 8.7% of the variance and was related to nutrient-poor dummy variables (Figure S2.2A). Then, based on the aggregations across the two main factors extracted (Figure S2.2B) we classified the forests as having clearly high or clearly low nutrient availabilities. The remaining forests, for which empirical evidence was insufficient to classify them as nutrient-rich or nutrient-poor or indicated moderate nutrient availability were classified as medium nutrient availability. To maximize robustness, we included only the forests with clearly high (N = 23) and clearly low (N = 69) nutrient availabilities in the main analysis, discarding data from the 37 remaining forests with medium nutrient availability. We also present the analysis with all the available data (including the medium nutrient availability category) in Figure S2.8 and in the Supplementary Models.

Statistical analyses

We ran generalized linear models (GLM) to test for differences in CUEe, NEP, Re and GPP between forests of high and low nutrient availability, accounting for the possible effects of GPP, mean stand age, management (as a binary variable: managed or unmanaged) and climate [MAT, MAP and water deficit (WD) = 1 – (AET/PET)*100]. That is, NEP ~ GPP + nutrient availability + Age + Management + MAT + MAP + WD. We tested for interactions up to the second order among GPP, nutrient availability, age and management. The significant variables of the final model (minimum adequate model) were selected using stepwise backward variable selection and the AIC of the respective regression models. To evaluate the variance explained by each predictor variable, we used the *averaged over orderings* method (the *lmg* metric, similar to hierarchical partitioning) to decompose R^2 from *R* (R Core Team, 2013) with the package relaimpo [Relative Importance for Linear Regression (Grömping, 2006)]. Finally, we tested whether nutrient status, management, age and climatic variables could lead to changes in patterns of biomass allocation with stepwise forward regressions. Model residuals met the assumptions required in all analyses (i.e., normality and homoscedasticity).

The robustness of our analyses was tested by five different methods: i) running weighted models using the inverse of the uncertainty of the estimates as a weighting factor, ii) using only data derived from eddy covariance towers, iii) restricting comparison of nutrient-rich and nutrient-poor forests to a common rank of GPP (GPP < 2500 gC m^{-2} $year^{-1}$ in Figures 2.1 and 2.2, thus excluding most of the tropical forests and using forests presenting GPPs above 1000 and below 2500 gC m^{-2} $year^{-1}$ in Figure S2.10), iv) using an alternative classification of nutrient availability (the second most plausible classification) as an analysis of sensitivity and v) using the factors extracted for the classification of nutrients as nutrient richness covariates instead of using the binary factor nutrient availability. Detailed information about the methods used in this paper is presented in Supplementary Information.

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Chapter 3

Nutrient availability and climate as the main determinants of the ratio of biomass to NPP in woody and non-woody forest compartments

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Abstract

Forest ecosystems accumulate large amounts of carbon in living tissues. The residence time of this carbon in the ecosystem depends largely on the turnover time of these tissues, which can be estimated as a surrogate of the ratio of biomass to net primary production (B:NPP). We used a global forest database of 310 sites containing data for biomass stocks and NPP to investigate the differences of B:NPPs among species and forest compartments and to determine B:NPPs main exogenous (mainly climate and nutrient availability) and endogenous (leaf habit and stand age) drivers. We used asymptotic exponential functions to adjust the B:NPPs of woody compartments to a theoretical stationary state to allow comparisons between forests of different ages. The B:NPPs of woody tissues (branches, stems, and coarse roots) were positively influenced by stand age, conversely to fine roots and leaves, which were weakly dependent on the age of the forest. The B:NPPs of woody tissues were positively correlated with nutrient availability, whereas fine-root B:NPPs decreased with increasing nutrient availability. The foliar B:NPP of evergreen forests was positively correlated with water deficit, and the fine-root B:NPP was correlated positively with the seasonality of precipitation and with annual thermal amplitude but negatively with water deficit. Our results support the influence of climate on the B:NPPs of non-woody compartments and identify nutrient availability as the main influence on the B:NPPs of woody tissues.

1. Introduction

Forest ecosystems accumulate and sequester large amounts of carbon, both as living tissues and as soil organic matter (Dixon et al. 1994; Myneni et al. 2001; Pan et al. 2011). The expected duration of a carbon atom in an ecosystem, however, strongly depends on the compartment of the forest to which the atom was allocated (e.g. foliage, stems, roots; Luo et al. 2003; Zhang et al. 2010). To evaluate this duration, ecologists often use the term “turnover time” as the inverse of “turnover rate” as defined by Margalef (1974). The study of turnover times of compartments is of paramount importance not only to determine the duration of carbon sequestration in the living biomass of an ecosystem, and therefore to properly assess carbon sequestration, but also to provide a better understanding of carbon and nutrient cycling in forests.

The turnover time of carbon, also termed mean residence time, indicates the average time that a carbon atom remains in its initial ecosystem compartment under the assumption of stationarity: the condition for which input (biomass production) equal output (necromass production) (i.e. biomass does not accumulate in the system). This assumption, however, is rarely realised (except for foliage and fine roots), so the study of turnover times has usually been based on modelling (Dewar 1991; Kicklighter et al. 1999; Barrett 2002; Luo et al. 2003; Karlberg et al. 2006; Zhang et al. 2010) rather than on empirical data. In this sense, the ratio of biomass to net primary production (B:NPP) may serve as a useful surrogate of the turnover times under determined conditions (stationarity or pseudo-stationarity).

Extensive research has focused on the B:NPPs of foliage (Reich et al. 1992; Aerts 1995; Wright and Westoby 2003) and fine roots (Dahlman and Kucera 1965; Nadelhoffer 2000; Gill and Jackson 2000; Majdi et al. 2005), but very few studies have analysed other living compartments such as branches, stems, or coarse roots, and, to the best of our knowledge, no single study has yet synthesised the turnover times of all compartments in concert.

The factors controlling the variability of B:NPPs of different compartments in forests under various environmental conditions (e.g. climate and nutrient availability) and endogenous characteristics (e.g. stand age and leaf habit) remain undetermined. Detecting the potential controls of B:NPPs of different compartments may help to predict the fate of carbon in different types of forests.

Endogenous factors such as stand age and leaf habit or type, combined with exogenous factors such as climate, nutrient availability, or management, can influence B:NPPs in different ways in different forest compartments. Nutrient-rich forests tend to have lower B:NPPs than nutrient-poor forests (Jordan and Herrera 1981) because of a higher biomass production, but this relationship has only been tested for foliage and fine roots (Reich et al. 1992; Aerts 1995; Ryser 1996). Nutrient-rich forests typically allocate a larger proportion of their photosynthates to aboveground biomass compared to nutrient-poor forests (Litton et al. 2007; LeBauer and Treseder 2008; Vicca et al. 2012; Fernández-Martínez et al. 2014a), and the ultimate effect of nutrient availability on carbon sequestration in ecosystems thus depends on the concerted response of the turnover times of the various plant organs and on the allocation strategy. Changes in carbon allocation may also lead to differences in carbon stocks in aboveground versus belowground compartments, depending on nutrient availability, and therefore to contrasting relationships between B:NPP and nutrient richness for aboveground versus belowground compartments. If these mechanisms were real, we would expect the relationship between B:NPP and higher nutrient availability to be positive in aboveground compartments and negative in belowground compartments.

The aim of this study was to calculate the B:NPPs of five compartments (foliage, branches, stems, coarse roots, and fine roots) of forest ecosystems around the world and to explore the endogenous (stand age and leaf habit and type) and exogenous (climate and management) factors that control them. We also particularly investigated the role of nutrient availability as a likely control of B:NPP.

2. Materials and Methods

2.1. Data collection

2.1.1 Global forest database

We updated and analysed a global forest database (Luyssaert et al. 2007) containing data from 1990 to 2012 for NPP and stand biomass for five forest compartments (foliage, branches, stems, coarse roots, and fine roots [diameter ≤2 mm]) from 310 sites around the world comprising boreal, temperate, Mediterranean, and tropical biomes, albeit the tropical and Mediterranean forests were less well represented. Only 80 of the forests provided the necessary data to calculate B:NPP for at least one of the compartments. The database also included descriptive information of the forests, such as stand age, leaf type (needleleaved, broadleaved, or mixed forest), leaf habit (evergreen, deciduous, or mixed forest), type of management (managed or unmanaged forests), and nutrient availability (see Fernández-Martínez et al., 2014; Vicca et al., 2012), which we used to calculate a proxy of *nutrient richness* (see section 2.2.3).

2.1.2 Climatic data

We extracted climatic data for our forests from the WorldClim database (Hijmans et al. 2005). This database provides suitable climatic data with a high spatial resolution (30 arc seconds, ca. 1 km at the equator) and contains robust mean monthly climatic data derived from a lengthy time series (1950 to 2000), including monthly temperature and precipitation and several other climatic variables such as annual thermal amplitude and seasonality of precipitation.

The time series for evapotranspiration (MOD16A2) from MODIS (Moderate Resolution Imaging Spectroradiometer) were downloaded for the period between 1 January 2000 and 27 December 2009 to obtain climatic proxies of potential and actual evapotranspiration (PET and AET, respectively). We downloaded the data with a resolution of 9 km^2 (3 × 3 km) around the central coordinates.

2.2. Data analyses

2.2.1 B:NPPs

We calculated B:NPP similar to previous studies (Dahlman and Kucera 1965; Margalef 1974; Malhi et al. 1999; Gill and Jackson 2000), dividing stand biomass by mean NPP for each compartment. The availability of NPP and biomass data was uneven for the compartments, so we calculated B:NPP for foliage, branches, stems, and coarse and fine roots from 71, 44, 44, 70, and 80 forests, respectively.

Stand biomass was strongly age-dependent in non-stationary compartments such as branches, stems, and coarse roots (Figure S3.1) (in contrast to foliage and fine roots). Previous studies have suggested a strong relationship between NPP and stand age (Carey et al. 2001 and references therein), but our data showed no clear trend with stand age (Figure S3.2). Nonetheless, comparing the biomasses or B:NPPs of forests of differing average stand ages (and thus biomasses) would be nonsensical for woody compartments. We avoided this problem and compared forests of different ages by adjusting the biomasses and B:NPPs of branches, stems, and coarse roots to their theoretical stationary state (at approximately 200 years of age, assumed to be when the percent annual increase in biomass and B:NPP from most compartments was <0.5%). We thus removed fast-growing species (e.g. *Acer* sp., *Alnus* sp., *Betula* sp., and *Populus* sp.) from the analyses. We calculated the stationary B:NPP by first fitting our data to an asymptotic exponential function, as conceptually suggested by Hougthon (2009). We then extracted the residuals of all cases and summed them to the predicted biomass or B:NPP of the function for 200 y (i.e. raw residuals + fitted B:NPP at 200 y). These adjusted values were used for regression models (see section 2.2.4) and to obtain means. The fitted value at 200 y only changed the means of the biomasses and B:NPPs, so our choice of age did not influence the significance of our results.

2.2.2 Climatic predictors

We used mean annual temperature (MAT) and precipitation (MAP) from the WorldClim database and calculated the length of the warm period (sum of months >5 °C) using mean monthly temperatures. We also extracted two key climatic variables: annual thermal amplitude (mean maximum minus mean minimum temperature for the year) and seasonality of precipitation (measured as the coefficient of variation of precipitation among months).

We calculated the percentage water deficit from the MODIS evapotranspiration time series as WD = (1 – [AET/PET])*100, (Fernández-Martínez et al. 2014b) as an indicator of the intensity of water stress the forests must withstand. We thus used eight climatic predictor variables: MAT, MAP, mean temperature and precipitation for the warm period, length of the warm period, annual thermal amplitude, seasonality of precipitation, and WD.

2.2.3 Assessment of nutrient availability

The forest database contained information about the nutrient status of the forests for variables such as soil type, texture, pH, nitrogen and phosphorous content, nitrogen mineralisation, C:N ratio, and CEC; foliar nitrogen and phosphorous concentrations; nitrogen deposition; and the history of the stand or explicit reports of the fertility of the forests (Vicca et al. 2012; Fernández-Martínez et al. 2014a), but information for all variables was not available for each site. We coded each variable into three levels of nutrient availability, high, medium, and low, following the methodology and data reported by Fernández-Martínez et al. (2014a). We then transformed each three-level factor into three dummy variables, each indicating high, medium, or low nutrient availability. We next performed a factor analysis to reduce the number of dimensions of our data set using only dummy variables indicating high and low nutrient availability. The first resulting factor (F1: nutrient richness covariate) explained 16% of the variance of the data and was correlated positively with nutrient-rich and negatively with nutrient-poor dummy variables.

2.2.4 Statistical analyses

We used stepwise forward regression models to correlate the B:NPPs (previously adjusted to the stationary state of 200 y) with the climatic variables (see section 2.2.2), the nutrient richness covariate (F1), management, and leaf type and habit. Predictor covariates were entered twice for selection in the models, with and without transformation to natural logarithms, to identify possible nonlinearities. The dependent variables usually required transformation to meet the assumptions of normality and homoscedasticity of the residuals. We evaluated the contribution of each predictor variable to B:NPP using the *PMVD* (Proportional Marginal Variance Decomposition, (Grömping 2007)) metric of the R (R Core Team 2013) package *relaimpo* (Grömping 2006) as a measure of the variance explained by each predictor. We excluded variables with high collinearity from the models (variance inflation factor [VIF] >5). We also used the three levels of nutrient availability (high, medium, and low) used by Vicca et al. (2012) and Fernández-Martínez et al. (2014b) to compare means among groups. Differences among groups were tested using ANOVA tables and the Tukey HSD test for multiple comparissons.

3. Results

3.1. Adjusted B:NPPs across forest types

A strong correlation between woody (branches, stems, and coarse roots) biomass and forest age (Figure S3.1) produced a strong correlation between woody B:NPPs and stand age (Figure 3.1). The asymptotic exponential functions indicated that branches reached a stable B:NPP of 45 y when trees were about 150 years old (Figure 3.1a). Stationary B:NPPs for stems and coarse roots reached 115 and 104 y, respectively, at an age of approximately 200 y (Figure 3.1b and c). The fitted functions between B:NPP and stand age presented a pseudo-R^2 of 0.31, 0.81, and 0.73 in branches, stems, and coarse roots, respectively (Figure 3.1). B:NPP and stand age were not significantly correlated for fine roots or evergreen foliage (Figure 3.2).

Stationary B:NPP did not significantly differ among biomes or leaf habits in woody compartments (ANOVA, $P > 0.05$) but differed significantly between leaf types for foliage and fine roots (ANOVA, $P < 0.01$; Table 3.1). Biome-averaged differences among woody fractions (branches, stems, and coarse roots), however, were large. The B:NPPs at the 20 and 80 percentiles were 21-80 y for branches, 71-171 y for stems, and 63-176 y for coarse roots. Differences among woody compartments were statistically significant for some species (Table 3.1). For example, *Fagus sylvatica* had a longer B:NPP in the stem (122 ± 19) and coarse-root (83.9 ± 18) fractions than in the branch fraction (22 ± 3, $P < 0.05$). This trend was also consistent for *Picea abies* and *Pseudotsuga menziesii* ($P < 0.05$ and $P < 0.01$, respectively). The B:NPPs of fine roots and leaves ranged between 1 and 5 y, with lower B:NPPs in deciduous than evergreen forests for both fine roots and leaves ($P < 0.01$, Table 3.1).

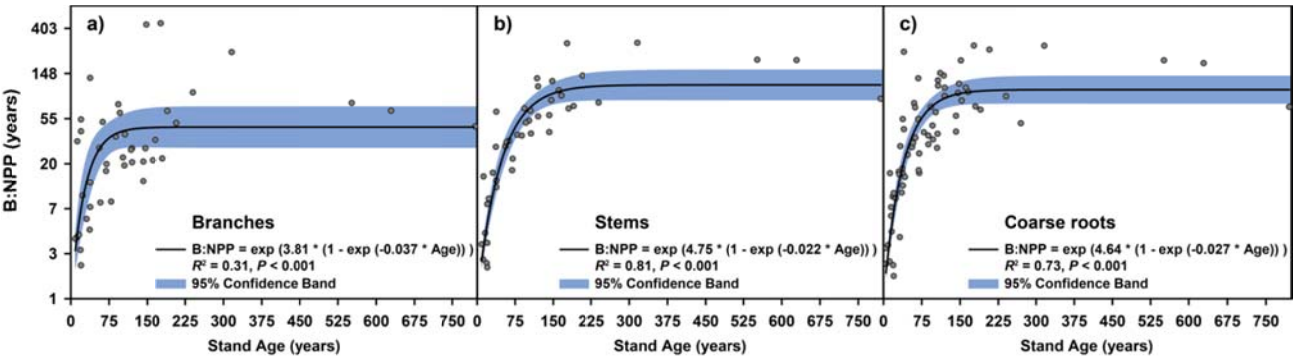

Figure 3.1: Relationships of the B:NPPs of a) branches, b) stems, and c) coarse-roots with stand age. Data were fitted using an asymptotic exponential function.

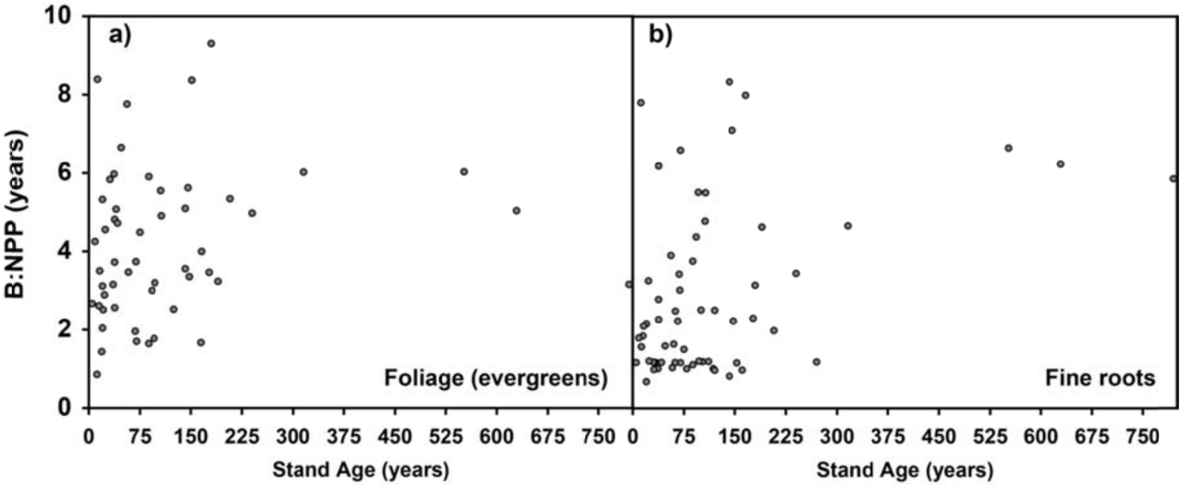

Figure 3.2: Relationships of the B:NPPs of a) foliage and b) fine roots with stand age.

Table 3.1: B:NPPs (mean years ± standard error) of leaves, branches, stems, and coarse and fine roots across species and biomes adjusted to the stationary state (200 y) (except for foliage and fine roots). The number of forests is shown in parentheses. Different letters within a column indicate significant differences among groups ($P < 0.05$) using Tukey's HSD test for multiple comparisons.

Species	**Foliage**	**Branches**	**Stems**	**Coarse roots**	**Fine roots**
Cocos nucifera	2.5 (1)				
Fagus sylvatica	1.1± 0.1 (12)	21.6 ± 3.0 (5)	121.9 ± 19.2 (5)	83.9 ± 17.6 (8)	1.1 ± 0.04 (8)
Larix gmelinii		20.9 (1)	100.2 (1)	37.0 (1)	
Picea abies	4.4± 0.4 (11)	31.3 ± 16.6 (5)	134.1 ± 31.1 (5)	86.1 ± 7.4 (7)	1.4 ± 0.4 (7)
Picea mariana	9.5 (2)			186.7 (2)	5.5 (1)
Pinus banksiana	2.0 (1)			277.9 (1)	3.4 (1)
Pinus ponderosa	4.1± 0.5 (13)	99.7 ± 35.2 (12)	118.5 ± 17.9 (12)	132.5 (2)	2.0 (2)
Pinus radiata	5.3 (1)		167.5 (1)	155.5 (1)	0.7 (1)
Pinus strobus				163.3 ± 18.1 (4)	
Pinus sylvestris	4.4± 1.1 (6)	134.7 ± 99.0 (3)	93.8 ± 23.1 (3)	124.2 ± 63.5 (3)	2.1 ± 0.6 (3)
Pinus taeda				167.4 (1)	
Pseudotsuga menziesii	3.5± 0.5 (12)	35.8 ± 5.6 (12)	94.0 ± 14.1 (12)	85.0 ± 15.6 (12)	6.1 ± 0.6 (11)
Biome					
Boreal evergreen	5.4± 1.1^{a} (9)	134.7 ± 99.9^{a} (3)	93.8 ± 23.1^{a} (3)	172.8 ± 36.9^{a} (6)	3.0 ± 0.7^{ab} (5)
Boreal deciduous	1.0^{b} (2)	20.9 ^{a} (1)	100.2 ^{a} (1)	37.0 ^{a} (1)	2.2 ^{ab} (1)
Temperate evergreen	4.1± 0.3^{a} (42)	99.5 ± 22.8^{a} (32)	139.7 ± 20.3^{a} (32)	132.5 ± 15.8^{a} (31)	3.7 ± 0.6^{a} (24)
Temperate deciduous	1.1± 0.1^{b} (15)	30.2 ± 7.7^{a} (7)	113.4 ± 14.7^{a} (7)	184.4 ± 89.8^{a} (12)	1.4 ± 0.2^{b} (12)

3.2. Controls of the B:NPPs

Our results indicated that the various forest compartments were correlated with different endogenous and exogenous factors (Table 3.2). Age-adjusted B:NPP was controlled by nutrients in the compartments that accumulate biomass over long periods (branches, stems, and coarse roots). Nutrient richness explained 20, 35, 9, and 6% of the variance in the B:NPPs of branches, stems, and coarse and fine roots, respectively. Foliage B:NPP was not correlated with nutrient availability ($P > 0.05$, Table 3.2). Nutrient-rich forests had longer B:NPPs than nutrient-poor forests in woody compartments (Tukey's test, $P < 0.05$; Table S3.2, Figure 3.3a, b, and c). The response of the B:NPPs of woody fractions to nutrient availability, however, differed from the response of the fine-root fraction (Table 3.2, Figure 3.4), which behaved oppositely (Table S3.2, Figure 3.3d). Forests with higher nutrient availability had longer B:NPPs in woody tissues (branches, stems, and coarse roots, Figure 3.4a) in comparison to nutrient-poor forests ($P < 0.05$), but forests with nutrient limitations had longer B:NPPs in fine roots than nutrient-rich forests ($P = 0.002$; Figure 3.4b). Nutrient availability was not aligned with old or young forests (ANOVA, $P > 0.1$). Our results should therefore not be biased because of age differences between nutrient-rich and nutrient poor forests. Despite the possible combined effect that nutrient availability and management can have on biomass production (Campioli et al. 2015), B:NPP did not differ significantly between managed and unmanaged forests in any compartment.

Carbon stocks in the biomasses of branches and stems increased with nutrient availability (Table S3.2, Figure 3.5; $P < 0.05$). Fine roots had the opposite trend, but the results were not statistically significant. Production (NPP) varied little (Table S3.2, Figure 3.5) among nutrient classes. The differences in B:NPP with nutrient availability were thus due to differences in stand biomass rather than to differences in NPP.

Leaf habit was responsible for the largest differences in foliage B:NPP (Table S3.2). Foliage B:NPP averaged approximately 1 y in deciduous forests and 4.3 ± 0.4 y in evergreen forests (Table S3.2). Branch B:NPP was longer in evergreen than deciduous forests (Table 3.2). Evergreen foliage

B:NPPs were positively correlated with water deficit, and fine-root B:NPPs were higher in forests with low water stress (Table 3.2). The seasonality of precipitation, however, was the most (positively) correlated variable with fine-root B:NPP, explaining 58% of its variance. High values of annual thermal amplitude were also correlated with high fine-root B:NPPs. Evergreen foliage and fine-root B:NPPs were marginally positively correlated with stand age (Figure 3.2, Table 3.2).

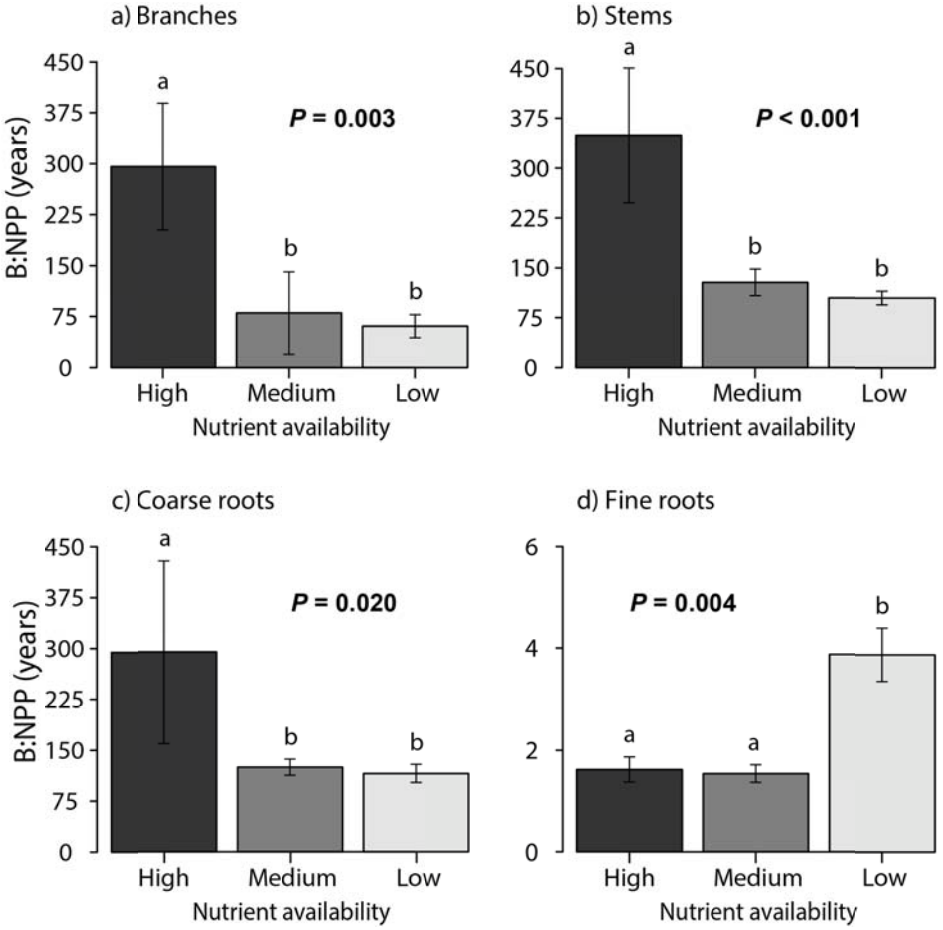

Figure 3.3: B:NPPs of a) branches, b) stems, c) coarse roots, and d) fine roots for different levels of nutrient availability. The branch, stem, and coarse-root B:NPPs have been adjusted to the stationary state (200 y) using the equations in Figure 3.1. Exact values can also be found in Table S3.2. Different letters above the bars indicate significant differences using Tukey's HSD test for multiple comparisons at the 0.05 level.

Table 3.2: Summary of the models for the B:NPPs of evergreen foliage, branches, stems, and coarse and fine roots using stepwise forward regressions. For these analyses, the B:NPPs of woody compartments were adjusted to 200 y using an asymptotic exponential function (see Materials and Methods). Values indicate β ± standard error, and the proportion of variance explained (in %) is shown in parentheses. For branches, stems, and coarse roots, the reported variance explained by stand age (*) is the R^2 from the asymptotic exponential functions in Figure 3.1 and is not accounted for in the R^2 of the stepwise models. All coefficients were significant at the 0.05 level except those marked with †, indicating significance at the 0.1 level, or by n.s., indicating that the term was not significant. PS, precipitation seasonality; ThA, annual thermal amplitude; WD, water deficit. Ln indicates natural-log transformation. For leaf habit, D indicates deciduous and E indicates evergreens.

Stepwise regression	**Ln foliage**	**Ln branches**	**Ln stems**	**Ln coarse roots**	**Ln fine roots**
Leaf habit		D < E (11)			
Nutrient richness (F1)		0.48 ± 0.15 (20)	0.59 ± 0.15 (35)	0.36 ± 0.14 (9)	-0.18 ± 0.09 (6)
PS					0.69 ± 0.08 (58)
Ln ThA				0.26 ± 0.14 (5)†	0.25 ± 0.10 (5)
Ln WD	0.30 ± 0.13 (8)				-0.23 ± 0.09 (4)
Ln Age	0.28 ± 0.13 (7)	(31)*	(81)*	(73)*	0.19 ± 0.08 (5)
***R*²**	15	31	35	14	78

4. Discussion

Our results identified large differences in B:NPPs among forest compartments but only small differences among tree species (Tables 3.1 and S3.2, Figure 3.3). The slow-growing species in this study may thus have similar structural and functional properties, which allowed us to compare the role of exogenous controls of B:NPP spatial variability. The B:NPPs were mostly driven by climate in non-woody tissues but by age and nutrient availability in woody tissues.

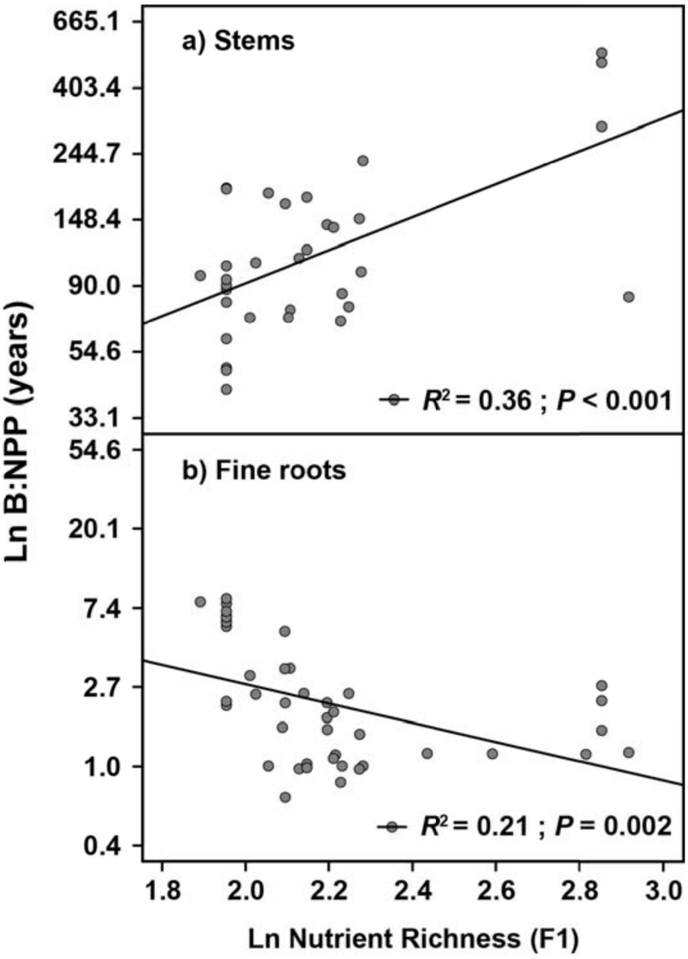

Figure 3.4: Relationships of the B:NPPs of (a) stems and (b) fine roots with nutrient richness.

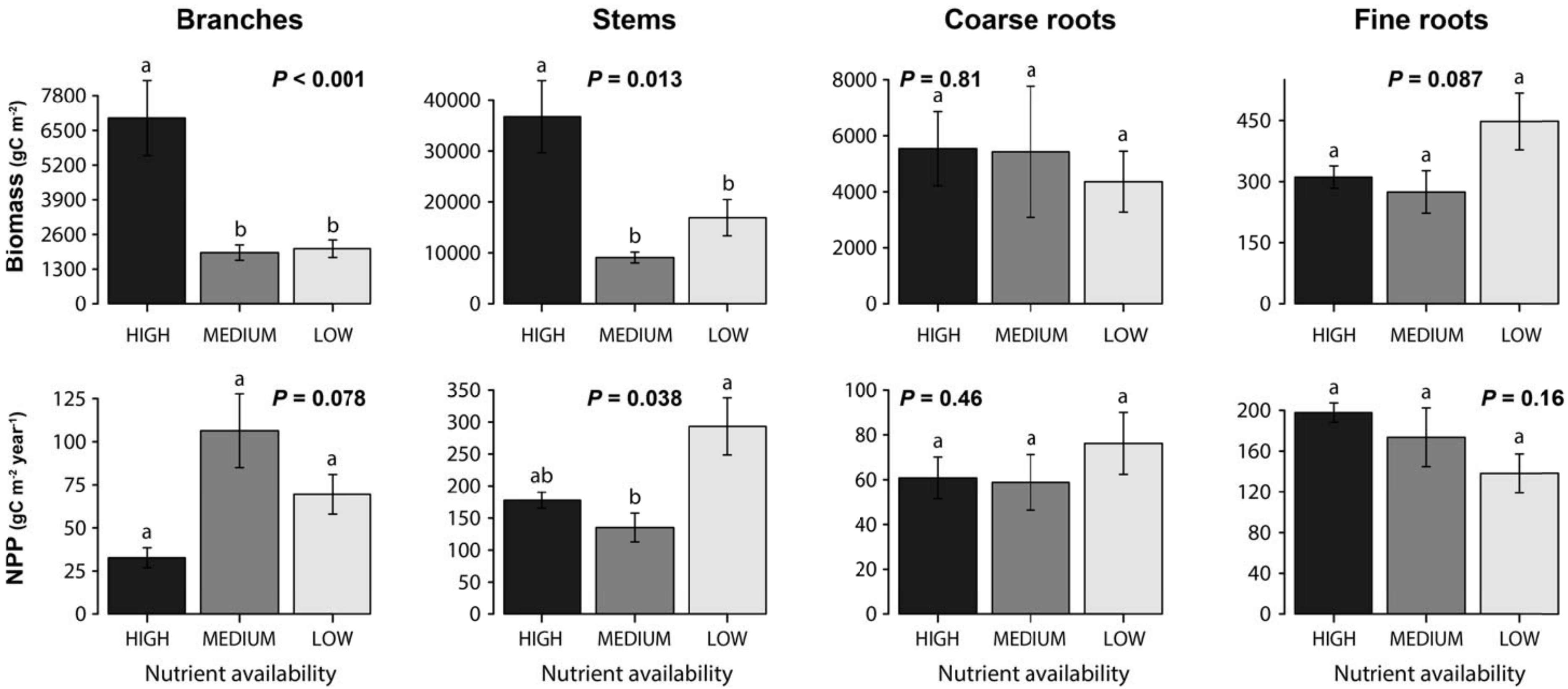

Figure 3.5. Stand biomass and NPP of branches, stems, coarse-roots and fine-roots for different levels of nutrient availability. The biomasses for branches, stems, and coarse roots have been adjusted to the stationary state (200 y) using an asymptotic exponential function (see Materials and Methods for further information). Exact values can also be found in Table S3.2. Different letters above the bars indicate significant differences using Tukey's HSD test for multiple comparisons at the 0.05 level.

4.1. The role of climate in non-woody compartments

Climate played a significant role in determining the B:NPPs of non-woody compartments such as foliage and fine roots but not of branches, stems, and coarse roots. The negative correlation between foliar B:NPPs and water deficit may indicate that forests under high water stress are unable to sustain as much leaf biomass as forests with good hydric conditions, as previous studies have reported (Fernández-Martínez et al. 2014b).

Fine-root B:NPPs were strongly correlated with intra-annual climatic variability (thermal amplitude and especially the seasonality of precipitation). This relationship supports previous findings, suggesting that the B:NPPs of fine roots decrease with climatic seasonality (Gill and Jackson 2000). Climatic seasonality may lead to periods of extreme weather (e.g. drought or cold) during the year that may kill fine roots, thus decreasing their life span. This negative effect of seasonality may also indicate that warmer forests (with less seasonality) need a higher fine-root NPP to sustain the same pools of fine-root biomass (Gill and Jackson 2000) due to the higher metabolic rates.

In contrast to foliage and fine roots, the B:NPPs of woody tissues were not correlated with climate, perhaps because woody tissues are organs that accumulate much biomass in a very recalcitrant form and are therefore relatively insensitive to meteorological conditions. Also, the turnover of woody tissues is primarily associated with tree mortality and is therefore less sensitive to normal meteorological conditions (without considering events of extreme weather causing disturbances such as windthrows, storms, or heat waves causing mass mortality).

4.2. The role of nutrient availability in woody and non-woody compartments

The positive effect of nutrient availability on woody B:NPPs was driven by the larger carbon pools in nutrient-rich than in nutrient-poor forests, not by an increase in biomass production (NPP), which remained fairly constant among the classes of nutrient availability (Table S3.2). We can thus infer that either necromass production is higher in nutrient-poor forests or that nutrient-rich forests can sustain more living biomass than nutrient-poor forests. This finding also supports the hypothesis that nutrient-rich forests allocate larger proportions of photosynthates to wood than nutrient-poor forests (Vicca et al. 2012). Woody compartments have longer B:NPPs than non-woody organs (Tables 3.1 and S3.2, Figure 3.3), so our findings also suggest that nutrient-rich forests are more likely to act as carbon sinks than nutrient-poor forests (Fernández-Martínez et al. 2014a). Nutrient-rich forests thus accumulate more biomass, and the carbon is more likely to reside longer in the living biomass.

In contrast to woody compartments, fine-root B:NPPs are shorter in nutrient-rich forests, supporting previous research suggesting that nutrient-poor forests increase the life-span of fine roots to increase nutrient-use efficiencies and thus to avoid nutrient losses (Reich et al. 1992; Aerts 1995; Ryser 1996). Foliar B:NPPs were not significantly correlated with nutrient richness, which may be linked to the hypothesised higher resorptive capacity of leaves than of fine roots (Freschet et al. 2010).

4.3. Methodological considerations

The non-stationarity of woody compartments that we have attempted to resolve by removing the effect of stand age from our estimates of B:NPP suggests that our results should be interpreted with caution. Turnover times in leaves and fine roots could theoretically be calculated as the pool-to-flux ratio, because biomass in these compartments reaches a steady state at relatively young ages (Ryan et al. 1997; Chen et al. 2007). This methodology (simple B:NPP calculation) to evaluate turnover times, however, may certainly underestimate the real average time that an amount of carbon will reside in compartments where biomass increases with time (i.e. branches, stems, and coarse roots), leading to a strong association between turnover time and age (Figure 3.1). Biomass in woody compartments, however, tends to a steady state with age (Hougthon 2009; Fernández-Martínez et al. 2014b), so the pool-to-flux ratio might provide reliable estimates in old-growth forests that have already reached a stationary state when the inputs equal the outputs (NPP – necromass production = 0). The alternative to studying only old-growth forests is to adjust the pool-to-flux ratio to a theoretical stationary state of the stands following an asymptotic function describing the increase in pool-to-flux ratio with age, which is the methodology we have chosen.

By adjusting B:NPPs to the stationary state, we can provide surrogates of turnover times that should be useful to forest managers and the modelling community.

5. Conclusions

We detected large differences in B:NPPs among forest compartments but only small differences among tree species (Tables 3.1 and S3.2, Figure 3.3). Once the effect of stand age was removed for compartments without stationary behaviour (Figure. 3.1), nutrient availability (Figure 3.4) and climate (mostly water deficit and seasonality) were identified as playing crucial roles in determining the B:NPPs of woody and non-woody tissues, respectively (Table 3.2).

Acknowledgements

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Chapter 4

Atmospheric deposition, CO_2, and change in the land carbon sink

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Abstract

Atmospheric CO_2 concentration continues to increase; but although atmospheric deposition of sulphur and nitrogen is increasing in developing regions, it has declined in Europe and the USA. Using flux time series for 23 forests, we found that site-level net ecosystem production and gross primary production have increased by 1% annually from 1995 to 2011. Increasing atmospheric CO_2 is the most important factor driving the increasing carbon sink strength in Europe and the USA. We also found that the reduction of sulphur deposition in Europe and the USA involves a higher recovery in ecosystem respiration than in gross primary production, limiting the increase of carbon sequestration. By contrast, trends in climate and nitrogen deposition barely contributed to changing carbon fluxes. Our findings support the hypothesis of a CO_2-fertilization effect in nature and suggest sulphur deposition plays a significant role in the carbon balance of forests in industrialized regions.

1. Main text

Terrestrial ecosystems are key components of the global carbon cycle. Since the 1960s, they have been sequestering, on average, 15–30% of the anthropogenic CO_2 emitted into the atmosphere (Canadell *et al.*, 2007; Le Quéré *et al.*, 2009). The increase in atmospheric CO_2 concentration (hereafter CO_2) affects the terrestrial biosphere in various ways: warming the climate (radiative effect) (Alexander *et al.*, 2013), increasing photosynthesis (CO_2 fertilization), decreasing transpiration by stimulating stomatal closure (leading to increased water-use efficiency) and changing the stoichiometry of carbon, nitrogen and phosphorus (C:N:P) in ecosystem carbon pools (Peñuelas *et al.*, 2013). Although all models simulate rising CO_2 making a significant contribution to increasing plant productivity and C storage, empirical evidence remains elusive (Ciais *et al.*, 2013). Observations of large-scale increases in productivity (or "greening") in the Northern Hemisphere have been attributed to the CO_2-fertilization effect (i.e., more CO_2 leads to more photosynthesis), lengthening of the growing season due to higher winter, spring or autumn temperatures (Linderholm, 2006), nutrient fertilization (Fernández-Martínez *et al.*, 2014b), recovery from acidic deposition(Büntgen *et al.*, 2013) and afforestation or forest regrowth(Pan *et al.*, 2011). However, some studies indicate that, after decades of increasing C sequestration, terrestrial productivity and C sinks are saturating (Büntgen *et al.*, 2013; Nabuurs *et al.*, 2013).

Many experimental studies have shown that productivity increases when ecosystems are exposed to artificially elevated CO_2(Ainsworth & Long, 2005), but in natural ecosystems at current CO_2 levels, a CO_2-fertilization effect has not yet been firmly established. Positive effects of increasing CO_2 on productivity are, in fact, only expected when other factors are not limiting growth (e.g., water and nutrient availability) (Peñuelas *et al.*, 2011). Some studies attributed increased ecosystem water-use efficiency to the reduction in transpiration resulting from increased CO_2 (Peñuelas *et al.*, 2011), but they have rarely been able to link it to enhanced plant photosynthesis (Keenan *et al.*, 2013).

Detecting a fertilization effect from increasing CO_2 in natural ecosystems is difficult because many other factors, that also alter ecosystem productivity trends, are changing concurrently. One obvious confounding variable is the physical change in climate, which alters ecosystem productivity, not only directly by impacting the ecosystem C cycle, but also indirectly by increasing nutrient mineralization rates and the length of the growing season. Atmospheric deposition of nitrogenous and sulphurous compounds (N and S deposition) can also alter ecosystem processes.

There is strong evidence indicating that N deposition has increased the terrestrial C sink (Magnani, 2002; Fleischer *et al.*, 2013; de Vries *et al.*, 2014; Fernández-Martínez *et al.*, 2014a). However, sustained high N, and especially S, deposition can also lead to acidification of forest ecosystems; this deposition negatively affects both plant productivity and soil processes (Oulehle *et al.*, 2011; Büntgen *et al.*, 2013). The role of S deposition on productivity and C storage, however, has rarely been explored (Oulehle *et al.*, 2011). In Europe and North America, air-quality policies to reduce emissions of pollutants (SO_2 and NOx) have proven effective and have decreased acidic deposition (mainly SO_4^{2-} and NO_3^-) substantially since 1980 (Menz & Seip, 2004; Lajtha & Jones, 2013). The reduction in acidic deposition of both N and S should lead to a slow recovery of forests to their normal functional states. On the other hand, reduced N deposition could also slow down forest growth and C sequestration once previously accumulated soil N is used up and N again becomes a limiting nutrient(De Vries *et al.*, 2009; Fernández-Martínez *et al.*, 2014a).

Here, we hypothesize that gross primary production (GPP), ecosystem respiration (Re) and the net C-sink strength (net land-atmosphere CO_2 flux or net ecosystem production, hereafter NEP have been accelerated during the last two decades by the increased temperature and CO_2, and the recovery from high loads of S deposition in Europe and North America. This process would have modulated the biogeochemical effects of rising CO_2. We tested this hypothesis at two spatial scales: locally (i.e., site level) and regionally (Europe and USA).

At local scale, we used long-term eddy-covariance observations of NEP, derived GPP, and Re from 23 temperate and boreal forest sites distributed across Europe and the USA (see Supplementary Figure S4.1**).** These forest sites were selected because they are the longest running flux sites with 10 or more years of data available between 1992 and 2013. The selected sites had no indication of major disturbances or management impacts on carbon fluxes (in contrast to the typical situation for grassland or cropland). For these 23 forests, we also used remotely sensed maximum leaf area index

(LAI) as a surrogate for forest growth, derived from the AVHRR GIMMS NDVI3g data set(Zhu *et al.*, 2013).

At the regional scale, we used gridded monthly NEP derived from the Monitoring Atmospheric Composition and Climate project (MACC) by atmospheric inversion from 1979 to 2013 (http://copernicus-atmosphere.eu) (Chevallier *et al.*, 2005), and atmospheric CO_2 from the observatories at Point Barrow and Mauna Loa (as the world reference for atmospheric CO_2 concentrations). Data for predictor variables were acquired from: i) gridded maps for wet N and S deposition for Europe (European Monitoring and Evaluation Programme) (European Monitoring and Evaluation Programme, 2013) and the USA (National Atmospheric Deposition Program)(National Atmospheric Deposition Program, 2013), and ii) historical climate data from the Climatic Research Unit (TS v.3.21) for time series of temperature, precipitation(Harris *et al.*, 2013) and the Standardized Precipitation-Evapotranspiration Index (SPEI) — a measure of meteorological drought (Vicente-serrano *et al.*, 2010). Because of the dense network of atmospheric monitoring stations needed for CO_2 flux inversion, and the large volume of N and S deposition data in Europe and the USA, we focused on these two regions (mapped in Figure S4.1).

Empirical evidence for CO_2 fertilization at the local scale

Averaged across the 23 temperate and boreal forests, annual NEP and GPP increased (mean ± SE) by 8.4 ± 1.8 and 11.2 ± 2.5 g C m^{-2} yr^{-1}, respectively, from 1992 to 2013 ($P < 0.005$). These increases represent relative annual increases of 1.1%, which are similar in magnitude to those reported in previous studies (Canadell *et al.*, 2007) and simulated by global models in response to rising CO_2 only (Sitch *et al.*, 2015). NEP increased over time in 18 of the 23 forests; for 11 of these 18 the increase was statistically significant at $P < 0.05$ (Figure 4.1a inset, Supplementary Table S4.1). Bootstrapping analysis indicated that forests with increasing NEP outnumbered those in which NEP did not increase ($P = 0.001$). Similarly, GPP increased over time in 14 of the 23 forests, with eight forests presenting statistically significant trends at $P < 0.05$ (Figure 4.1b, Supplementary Table S4.1). Because NEP increased slightly less rapidly than GPP, Re of individual forests increased by 2.9 ± 2.5 g C m^{-2} yr^{-2}, but this signal was not statistically significant ($P = 0.25$) (Figure 4.1c and Supplementary

Table S4.1). A regression analysis of the individual trends (see Supplementary Information and Figure S4.2) indicated that the annual trends of NEP and GPP were mostly positively correlated with the increasing trend of CO_2. Forests with large standing biomass presented more positive trends in GPP and especially Re but not in NEP. Within forests, Re decreased with increasing mean annual temperature (MAT), age of the stand, and S deposition rate (Supplementary Information). Our analysis did not show significant statistical associations between C flux trends in individual forests and other possible factors or site characteristics, such as mean annual precipitation, mean annual N wet deposition, soil pH, or leaf type and habit.

NEP and GPP followed the same pattern when tested for the relative contribution of the trends for each season. Both NEP and GPP increased significantly in spring, in autumn and especially in summer (Figure S4.3, Table S4.2), i.e., during seasons when GPP exceeds Re. This finding suggests that the observed annual increases in NEP reported above were mainly driven by increasing rates of photosynthesis. Re also increased marginally during spring and in summer, indicating that trends in forest NEP were driven by larger changes in GPP than in Re, as found in previous syntheses (Luyssaert *et al.*, 2007; Schwalm *et al.*, 2012). Maximum LAI derived from satellite data exhibited an overall increasing trend (0.019 ± 0.007 m^2 m^{-2} yr^{-1}, $P = 0.003$) across the 23 forests (Supplementary Figure S4.4).

Across the 23 forests in Europe and USA, CO_2 increased, on average, by 2.0 ± 0.1 ppm yr^{-1}, but climate did not change significantly during the study period: neither MAT, nor the hydric conditions (SPEI) changed (Table S4.3). This climatic stability may partly result from the observed slowdown of increases in global and northern temperatures recorded during the last decade, the temperature hiatus(Kosaka & Xie, 2013), and the relatively short time series analysed (10 to 20 years). Conversely, N and especially S deposition did exhibit strong and in generally monotonic downward trends from 1995 to 2011 across the 23 forests. On average, N deposition decreased by 0.9% annually (-0.15 ± 0.04 kg N ha^{-1} yr^{-2}; $P < 0.001$) and S deposition by 5.0% annually (-0.26 ± 0.03 kg S ha^{-1} yr^{-2}; $P < 0.001$) (Table S4.3).

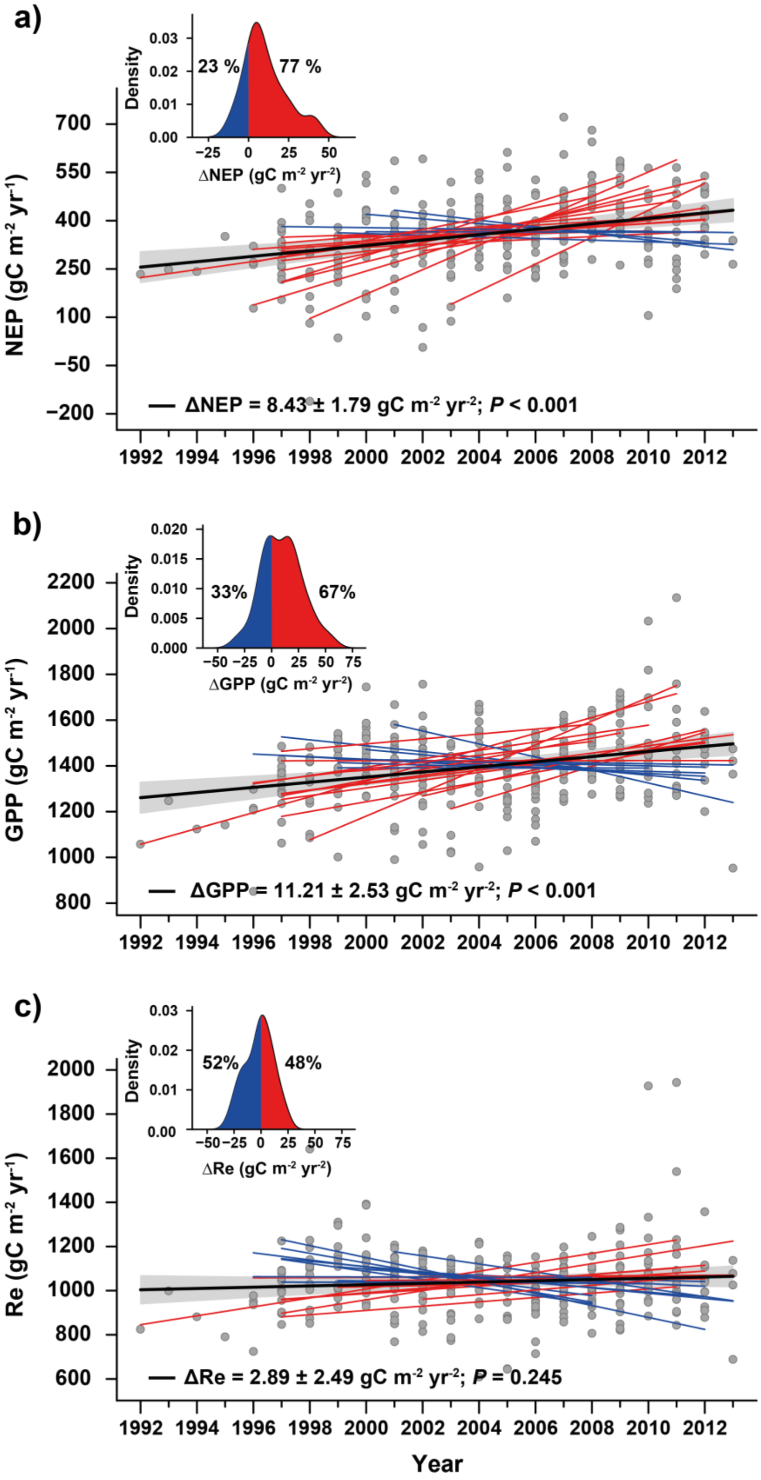

Figure 4.1: Long-term trends in carbon fluxes for 23 forests (1992–2013). Most of the forests presented increasing trends in a) NEP and b) GPP, whereas c) respiration remained fairly constant. The percentage of forests with increasing NEP was statistically higher (*P* = 0.001) than the percentage of forests with decreasing NEP, but the percentage of forests where GPP tended to increase was not statistically different (*P* = 0.28) from those with decreasing GPP. Red and blue lines indicate forests with increasing and decreasing trends, respectively, and black lines indicate the average slopes. The shaded area indicates the standard error of the average slope. Grey dots indicate site-year observations, and all values were adjusted to the same mean to remove site-specific variability. The inset shows the modeled distribution of the trends using kernel-density estimation, indicating the percentage of forests with increasing and decreasing trends. See Methods for further information on the methodology used to calculate the trends. All data came from eddy-covariance towers.

We used generalized linear mixed models (GLMMs) and multi-model inference(Burnham, 2004) to attribute the temporal trends of NEP, GPP and Re to changes in CO_2, N and S deposition rates, MAT, SPEI, LAI and their interactions. For each predictor (e.g., temperature), we also included an interaction term between the climatological average value (see Methods) of a given predictor for the site and the anomaly for the year to account for different effects of the predictors, depending on the mean for the site (see Methods for further details on model adjustment). This technique allowed us to disentangle the temporal effect of one single predictor, while taking into account the variance shared with the other predictors. The principal result of this statistical analysis is that increasing CO_2 appears to be the only predictor systematically associated with the observed increase in both NEP and GPP over time (Figure 4.2). For each ppm increase in atmospheric CO_2 concentration, NEP and GPP increased by 4.77 ± 0.47 and 4.75 ± 0.52 g C m^{-2} yr^{-1}, respectively. Conversely, increasing CO_2 had no statistically significant association with increasing Re (Figure 4.2b, Table 4.1 and Supplementary Information) despite the normally close relationship between Re and GPP (Fernández-Martínez *et al.*, 2014a). The statistical models further indicated that the decrease of N and S deposition during the period of flux measurements at both European and USA sites (Figure 4.3a and Table S4.3) has also affected the CO_2 fluxes in these forests.

The reduction in S deposition was associated with a net decrease in NEP (NEP sensitivity: 9.26 ± 3.48 g C m^{-2} yr^{-1} for each kg S ha yr^{-1}), likely because of a larger ($P = 0.011$) increase of Re than GPP as the forests recover from previous S deposition. Sensitivities are, -40.91 ± 7.99 and -22.30 ± 4.08 g C m^{-2} yr^{-1} for each kg S ha^{-1} yr^{-1}, for Re and GPP respectively. These results imply that, at the site level, the reduction in S deposition offset the positive effect of increasing CO_2 in NEP by 24 ± 9%.

The reduction in N deposition was associated with marginally reduced Re, but not with reduced GPP. Sensitivities are 26.10 ± 16.97 and 9.20 ± 7.10 g C m^{-2} yr^{-1} for each kg N ha^{-1} yr^{-1}, for Re and GPP respectively (see Figure 4.2b and Table 4.1). Hence, the positive effect of reducing S deposition for GPP and Re was offset because of reduced N deposition by 15 ± 11 and 38 ± 23%, respectively. Using past N and S deposition (the cumulative totals of the last 5 years) did not improve our models according to the second-order Akaike Information Criterion (AICc) and the Bayesian Information

Criterion (BIC). Trends in climate (MAT and SPEI) did not appear to influence trends in CO_2 fluxes over the timeframe of this study (1995–2011). Using temperature and SPEI from the warm half of the year (April – September) in our models did not confer a greater influence of climate on C flux trends (see Models 2.3.1 – 2.3.3 in Supplementary Information). Also, the increasing LAI (which is related to forest growth) was not correlated with the changing C fluxes (Figure 4.2). In addition, the model assessing potential causes for the increase in observed LAI detected increasing CO_2 as the only significantly contributing factor across the 23 forests (Figure S4.4b).

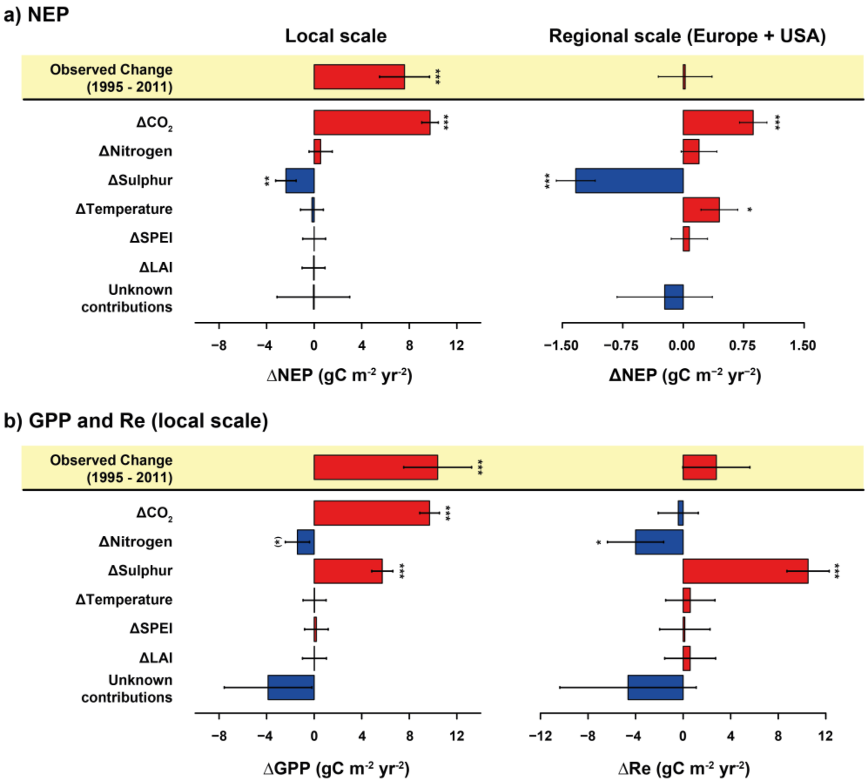

Figure 4.2. Temporal contribution of the predictor variables on (a) NEP and (b) GPP and Re, for the period 1995 – 2011. Models (see Supplementary Information, Models 2.1.1–2.1.3) suggest that increasing CO_2 is the main contributor to the observed increases in NEP and GPP. The difference between the modelled contributions and the observed trends (yellow shaded) has been considered as an unknown contribution to the temporal variation in C fluxes. The temporal variations of the predictors are shown in Figure 4.3. Error bars indicate standard errors. Units are ppm for CO_2, kg ha^{-1} yr^{-1} for S and N deposition, °C for temperature and standard deviation for SPEI. Local scale data came from eddy-covariance towers. Regional NEP data came from the MACC-II (Chevallier *et al.*, 2005). Error bars indicate standard errors. See Methods for information about the methodology used to calculate the contributions. All data came from eddy-covariance towers. Significance levels: $^{(*)}$, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The carbon balance (NEP as estimated by the MACC CO_2 atmospheric inversion) did not show any significant trend (Figure 4.2a) over Europe and the USA. While the seasonal analysis showed that NEP decreased during winter and autumn (i.e., smaller CO_2 uptake, Figure S4.3a), NEP significantly increased during spring and summer (i.e., larger CO_2 uptake). In contrast to the local analysis, temperatures and the occurrence of droughts (negative SPEI) significantly changed over Europe and the USA (Figure 4.3b, Table S4.3). This may suggest that the 23 sites are not a representative sample of the continental climate trends. Nonetheless, the regional GLMM analysis based on grid cells also indicated a clearly positive effect of CO_2 on NEP, increasing by 0.45 ± 0.09 g C m^{-2} yr^{-1} per ppm rise in CO_2. Annual S and N deposition rates tended to decrease from 1995 over Europe and the USA, by 0.02% yr^{-1} and 2.63% yr^{-1}, respectively ($P < 0.001$, Figure 4.3b, Table S4.3). The reduction in S deposition is again noted as having a negative impact on C sequestration (8.86 ± 1.69 g C m^{-2} yr^{-1} for each kg S ha^{-1} yr^{-1}) because of a possible greater stimulation of Re than GPP, as shown by the local analysis (Figure 4.2 and Table 4.1). The models suggested that NEP did not increase because the positive CO_2 effect was offset by a negative S effect by -154 ± 41%. Despite being a significant factor explaining spatial and interannual variability in NEP (see Model 2.2 in Supplementary Information), trends in N deposition had no significant impact on the trends of NEP. At first glance, our results on the impact of N deposition appear to disagree with the vast body of literature showing a positive effect of N deposition on biomass production and carbon sinks (Magnani *et al.*, 2007; De Vries *et al.*, 2009; Fernández-Martínez *et al.*, 2014a). But in fact, they agree with these studies. The regional model for derived NEP (Model 2.2 in Supplementary Information) shows that, spatially, the higher the N deposition, the stronger the effect of CO_2 fertilization on NEP (i.e., a positive interaction between mean N deposition and CO_2). The same model also shows that the lower the mean N deposition, the stronger the positive effect of increasing N deposition loads (negative interaction between mean and anomalies in N deposition). Nonetheless, as shown in Figure 4.2, the temporal decline in N deposition over Europe and the USA has reduced GPP and Re fluxes. Because the reduction of N deposition entailed a larger decrease in Re than in GPP, the net effect on NEP was marginally positive (see also sensitivities in Table 1). Given that the positive relationship between N deposition and GPP saturates at high values of N deposition(Fleischer *et al.*, 2013), these results may emerge from the fact that Europe and the USA were regions with relatively high N deposition loads. Our statistical models also inferred a positive but small effect of increasing temperature on NEP (7.76 ± 3.96 g C m^{-2} yr^{-1} K^{-1}, Table 4.1).

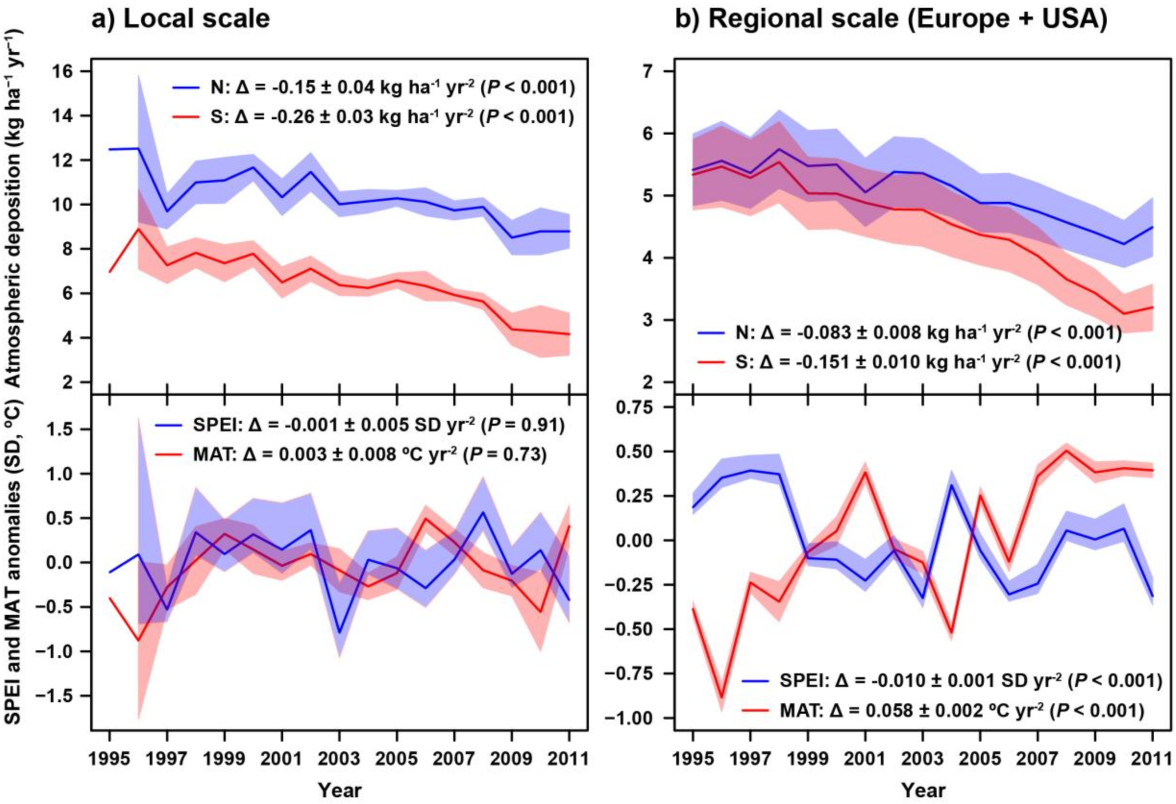

Figure 4.3. Temporal evolution and trends in N and S deposition, mean annual temperature (MAT), SPEI and CO_2 concentrations for (a) the local and (b) the regional scales (1995-2011). Trends were calculated using GLMMs with random slopes, with the forest or the pixel as a random effect and year as a fixed effect. Models also used an ARMA (1,0) autocorrelation structure. Shading indicates the 95% confidence intervals of the means (calculated as 1.96 times the standard error of the mean). See Methods for further details.

In combination with the increasing anthropogenic emissions of CO_2, this CO_2-fertilization effect observed over Europe and the USA is in line with the increase in annual amplitude of atmospheric CO_2 concentrations in the Point Barrow time series(Graven *et al.*, 2013) (Supplementary Figure S5) and the increasing C sink uptake as measured by the SCC index (see Methods for definition) during spring (Supplementary FigureS6).

Even though our statistical analyses do not directly prove causality, these results provide consistent empirical evidence that the CO_2-fertilization effect is the dominant explanatory variable of current NEP trends at local and regional scales (Figure 4.2). The results also indicate an important effect given the somewhat short span of CO_2 increases in our data set (increasing by 13–47 ppm during the study period, depending on the forest and database, see Supplementary Tables S4.1 and S4.3). This increase is relatively small compared to the increases applied in free-air CO_2-enrichment experiments (typically 475–600 ppm(Ainsworth & Long, 2005), i.e., a step change in CO_2 of ~100–200 ppm). Increasing CO_2 can enhance photosynthesis by increasing the rate of carboxylation and reducing photorespiration (Aber *et al.*, 2001). Increasing CO_2 might also decrease stomatal conductance under unstressed hydric conditions, leading to increased water-use efficiency(Prentice *et al.*, 2000; Keenan *et al.*, 2013), but the translation of this increase in water-use efficiency into higher plant growth and net carbon uptake in ecosystems is still controversial (Peñuelas *et al.*, 2011; Keenan *et al.*, 2013). Moreover, the increase in litter C:N ratio due to increased CO_2 (Sardans *et al.*, 2012) might reduce heterotrophic respiration(Hu *et al.*, 2001; Norby *et al.*, 2001) and, therefore, increase C-sink strength, at least during a transient period. Elevated CO_2, though, could also lead to higher root exudation, which could increase the priming effect and reduce soil C stocks (Bengtson *et al.*, 2012). Nonetheless, despite possible counteracting mechanisms, elevated CO_2 seems to be responsible for the increases in terrestrial photosynthesis and C sequestration during the last decades.

Recovery from high loads of acid deposition in Europe and USA

The negative effect of the reduction in S deposition on NEP was mediated by a stronger recovery of Re than of GPP from previously high rates of acid deposition (Figure 4.2 and Table 4.1). We interpret these results with the chain of processes during recovery from soil acidification. A reduction in S deposition typically increases soil pH, which, in turn, increases microbial activity (Andersson & Nilsson, 2001), thereby increasing heterotrophic respiration and nutrient mineralization and availability (Truog, 1947), with implications for both GPP and Re. The increase in pH and nutrient availability, in a recovery phase after high S deposition, can enhance photosynthesis(Field *et al.*, 1983). While higher microbial activity in response to reduced S deposition increases respiration, the associated higher nutrient availability does in turn reduce C allocation to root symbionts (Vicca *et al.*, 2012) and to free living heterotrophs via exudates, therefore ultimately reducing heterotrophic respiration (Janssens *et al.*, 2010). These two opposing mechanisms may compensate each other to some degree. Given the positive effects on Re, our results indicate a higher overall increase in microbial activity in ecosystems recovering from high S deposition loads than indirect effects from decreased belowground carbon allocation.

Table 4.1. NEP, GPP and Re mean sensitivity to predictors for (a) the local scale and (b) NEP sensitivity for the regional scale, for the period 1995 – 2011. Sensitivities (units of change in the response variable for each unit of change in the predictor) were calculated by dividing the temporal contributions of the predictor (Figure 4.2) by the trend of the predictors (Figure 4.3, Table S4.3). Nitrogen and sulphur refers to atmospheric deposition, and temperature to mean annual temperature. Errors were calculated by error propagation. NEP, GPP and Re units are g C m^{-2} yr^{-1}. Bold type indicates statistically significant sensitivities.

a) Local scale (23 sites)

	NEP	*P*	GPP	*P*	R_e	*P*
CO_2 (ppm)	**4.77 ± 0.47**	<0.0001	**4.75 ± 0.52**	<0.0001	-0.20 ± 0.82	0.4050
Nitrogen (kg ha^{-1} yr^{-1})	-3.55 ± 6.41	0.2921	9.20 ± 7.10	0.1025	26.10 ± 16.97	0.0673
Sulphur (kg ha^{-1} yr^{-1})	**9.26 ± 3.48**	0.0062	**-22.30 ± 4.08**	<0.0001	**-40.91 ± 7.99**	<0.0001
Temperature (K)	-66.87 ± 390.88	0.4327	12.13 ± 346.39	0.4861	214.44 ± 958.43	0.4122
SPEI	4.90 ± 1546.26	0.4988	-301.18 ± 3058.40	0.4611	-230.74 ± 3952.82	0.4769
LAI	-2.55 ± 46.62	0.4784	1.12 ± 48.84	0.4909	28.62 ± 104.33	0.3929

b) Regional (Europe + USA)

	NEP	*P*
CO_2 (ppm)	**0.45 ± 0.09**	<0.0001
Nitrogen (kg ha^{-1} yr^{-1})	-2.37 ± 2.70	0.1935
Sulphur (kg ha^{-1} yr^{-1})	**8.86 ± 1.69**	<0.0001
Temperature (K)	**7.76 ± 3.96**	0.0298
SPEI (SD)	-7.95 ± 23.53	0.3689

It has been suggested that the solar dimming effect, associated with, among other factors, sulphate aerosols, enhances photosynthesis due to an increase in the diffuse light fraction (Mercado *et al.*, 2009). A reduction in sulphur emissions reduces diffuse light (brightening effect) and therefore may contribute to constraining GPP. However, the negative effect of S deposition on GPP suggests that the possible benefit from increasing the diffuse light fraction because of sulphur emissions is lower than the negative effect of acidifying an ecosystem.

The impacts of N deposition on forest ecosystem C cycling have been widely studied. Reduced heterotrophic respiration is a general response to N deposition (Janssens *et al.*, 2010), possibly through an enhanced stabilization of soil organic matter, altered plant carbon allocation patterns and shifts in the saprotrophic community (Janssens *et al.*, 2010). It has also been suggested that the deposition of N, where it is a limiting nutrient, will increase net primary production (Luyssaert *et al.*, 2010) through its effect on photosynthesis(Field *et al.*, 1983) and possibly by the above-mentioned changing C allocation from symbionts and exudates to wood production (Vicca *et al.*, 2012). Nitrogen deposition can thus enhance ecosystem C uptake (or increase C stocks) (De Vries *et al.*, 2009; Fernández-Martínez *et al.*, 2014a). Our analysis of spatial variability supported these findings (Supplementary Information, Models 2.1 and 2.2), but our analysis of temporal variability indicated that decreasing N deposition had almost no effect on the trend in NEP, probably by equally reducing Re and GPP (Figure 4.2b). The widespread decrease in N deposition in Europe and the eastern USA, combined with an increase in CO_2 may have triggered a progressive negative feedback between reduced N mineralization and increased C:N ratios in plant litter, thus altering the C balance: i.e., as CO_2 increases, plant C:N ratios increase, hampering microbial soil activity and therefore N mineralization; this process further reduces the amount of N available to plants and increases the C:N ratio even more. This reasoning also agrees with previous studies suggesting that the fertilization effect of CO_2 is also possible in extremely N-limited ecosystems (Prentice *et al.*, 2000). The effect of CO_2 fertilization on GPP, however, may be stronger when other factors (e.g., nutrients) are not limiting growth (Kicklighter *et al.*, 1999).

Small effects of decadal-scale climate change on the carbon balance

The changing climate had different effects at different scales. Locally (23 sites), temperatures and drought did not significantly change and therefore could not be responsible for trends in C fluxes. However, NEP was found to be statistically positively associated with increasing temperature in the regional analysis. The estimated effect of temperature and drought on CO_2 fluxes, however, was clearly small compared to the effects of increasing CO_2 and decreasing S deposition. These results suggest that, to date, availability of CO_2 and nutrients and stoichiometric changes may have exerted a stronger impact on the terrestrial C balance than the changing climate (Fernández-Martínez *et al.*, 2014b). Nonetheless, given the slight increase in temperatures and droughts in our data sets, we cannot discard the possibility that climate change might have larger effects on C fluxes in the long term. Longer time series will be necessary to correctly answer this question.

Changing land carbon sinks

Global change is affecting the C budget of terrestrial ecosystems in several ways. Increasing CO_2 has increased the land C sink by enhancing GPP and limiting ecosystem respiration, and changes in S and N deposition rates are severely altering the C balance by enhancing or reducing photosynthesis and ecosystem respiration. It is far from certain whether terrestrial ecosystems will continue to respond positively to increasing CO_2, will saturate, or will eventually reach a tipping point beyond which respiration and the release of greenhouse gases exceed production. Stoichiometric imbalances and the limitation of key nutrients such as nitrogen and phosphorus (Peñuelas *et al.*, 2013; Fernández-Martínez *et al.*, 2014b) may already be acting as limiting factors for enhanced C sequestration.

2. Methods

Data sets

Carbon fluxes

We downloaded Level-4 CO_2 flux data collected by eddy-covariance towers from the Euroflux (GHG-Europe) and Ameriflux databases. When Level-4 data were not available, we downloaded gap-filled Level-2 data and checked for the homogeneity of the time series. In all cases, time series were either Level-2 or Level-4. We also used a global forest database updated in 2013 with data to 2010 (Fernández-Martínez *et al.*, 2014b) to obtain ancillary data (e.g., stand age and standing biomass) and for comparing CO_2 flux measurements. We selected 23 forests for which at least 10 years of CO_2 flux measurements were available. All forests were in the Northern Hemisphere between 39 and 68 °N (see Supplementary Figure S1), and the years of measurement ranged from 1992 to 2013. We also extracted information for all forests about leaf type and habit, and the age of the stand at the time of the measurements. Soil pH was extracted from the ancillary data of the forests when possible, or when not available, pH was assessed using data from the Harmonized World Soil Database (Nachtergaele & Velthuizen, 2010) and published literature reviews (Fernández-Martínez *et al.*, 2014b).

We gathered monthly NEP data on a global scale for 1979–2013 from the MACC (Monitoring Atmospheric Composition and Climate) CO_2 (http://copernicus-atmosphere.eu/) (Chevallier *et al.*, 2005) database. We chose this inversion model because it is one of the few that covers the last 30 years and, more importantly, because this inversion adjusts fluxes in each grid cell. The MACC CO_2 atmospheric inversion system, version 13r1, relied on the variational formulation of Bayes' theorem to analyse direct concentration measurements made at 130 sites over the globe between 1979 and 2013. Optimized fluxes were computed at global horizontal resolution $3.75^o \times 1.875^o$ (longitude, latitude) with a temporal resolution of eight days, separately for daytime and night time. The underlying transport model was run with interannually-varying meteorology from the ECMWF ERA-Interim reanalysis. Prior information for natural land vegetation, biomass burning, and ocean fluxes had no interannual variations at the annual scale, but fossil fuel fluxes did. This arrangement means that the interannual variations in the inverted total annual fluxes were fully driven by the measurements and the analysed meteorology.

Remotely sensed LAI data

We calculated the maximum annual LAI for the 23 forests derived from the AVHRR GIMMS NDVI3g data set (Zhu *et al.*, 2013) at 15-day intervals and 8-km spatial resolution for July 1981 to December 2011. The principles used for the generation of the LAI data set were based on the use of neural networks that were first trained with data from the overlapping GIMMS NDVI3g and MODIS LAI products. The trained neural network algorithm was then applied using the land-cover class, the latitude and longitude coordinates and the NDVI3g as the input data to generate the full temporal coverage of the GIMMS LAI data set. Further details are given by Zhu et al. (2013)

Climate and weather data

We extracted the climatological mean annual temperature and precipitation (MATc, MAPc) for all forests from the WorldClim database. We also used the CRU TS3.21 data set (Harris *et al.*, 2013) from the Climatic Research Unit to extract temperature and precipitation time series for our sites. In addition, the SPEI (Vicente-serrano *et al.*, 2010) from the global SPEI database (http://sac.csic.es/spei/database.html) was used as a measure of drought intensity. Annual means of temperature (MAT), precipitation (MAP) and SPEI were calculated for each year.

Atmospheric CO_2 concentrations

We used CO_2 concentrations recorded by eddy-covariance towers above the canopies of the forests when available. Annual atmospheric CO_2 records, however, sometimes contain implausible values because of gaps along the time series (years with lower CO_2 concentrations than the year before or higher than the next year's). We deleted the erroneous annual values and where possible filled the gaps using generalized additive models (GAM), adjusting a smoothing function. When this procedure was not possible, we used atmospheric CO_2 concentration data from the Mauna Loa observatory, provided by the Scripps Institution of Oceanography (Scripps CO_2 program). We also downloaded Point Barrow's time series of atmospheric CO_2 concentrations (provided by NOAA, Earth System Research Laboratory and Global Monitoring Division: http://www.esrl.noaa.gov/gmd) to check for changes in the seasonal amplitude of CO_2 concentrations (Graven *et al.*, 2013) and the SCC index up to 2013 in the Northern Hemisphere. The SCC index is calculated as the difference between the CO_2 concentrations in the first week of May and in the last week of June; it provides information on the carbon sink strength during spring, which is when maximum photosynthetic rates occur. Segmented regressions were also used to check for breaks in the trends.

Deposition data

Data for N ($NO3^-$ + $NH4^+$) and S ($SO4^-$) wet deposition were extracted from the European Monitoring and Evaluation Programme (EMEP), the MSC-W chemical transport model developed to estimate regional atmospheric dispersion and deposition of acidifying and eutrophying compounds (N, S) over Europe and the National Atmospheric Deposition Program (NADP) covering the USA. We used only data for wet deposition because the NADP database did not contain records for dry deposition. Analysis using N and S deposition data were restricted to Europe and the USA because temporal gridded maps of atmospheric deposition were not available for other regions. For the regional analyses, atmospheric deposition maps were adjusted to the resolution of the C flux maps (3.75 and 1.875° for longitude and latitude, respectively).

Statistical analyses

To test whether GPP, Re, NEP, NEP, LAI, N and S deposition, MAT and SPEI had changed during the study period, we first analyzed the individual (for each forest or pixel) annual time series of each of these variables and the seasonal time series of NEP, GPP and Re (e.g., NEP for winter is the sum of January, February and March NEP). The trends were extracted using the Theil-Sen slope estimator that minimizes the influence of extreme values (the breakdown point is ca. 29%) when calculating the slopes (mblm package (Lukasz Komsta, 2012) in R statistical software). This analysis has proven to be robust against temporal autocorrelation, non-normality and heteroscedasticity and produces results very similar to those of ordinary least squares regressions when errors are normally distributed and no outliers are present. We used two approaches to detect overall increases in NEP, GPP and Re: i) the z-transformed combined probability (Whitlock, 2005) to test the statistical significance of the alternative hypothesis of positive slope across all forests (*P* at individual sites was calculated using the alternative hypothesis of slope >0), and ii) *t*-tests determined whether the slopes were significantly greater than zero. Kernel densities were estimated to illustrate the proportions of forests with increasing and decreasing trends. We also tested the trends in the studied variables using mixed models with random slopes (e.g., NEP ~ year) where the forest or the pixel was the random factor (affecting the slopes of the year). Models also accounted for temporal autocorrelation using an autoregressive moving average (ARMA) (p = 1, q = 0) correlation structure. The main trends shown were calculated using this methodology.

We next tried to account for the variability among forests (N = 23) in the slopes of NEP, GPP and Re using weighted linear models (weighting for the number of observations for each forest) and stepwise forward model selection. The predictor variables we tested were climate (MATc and MAPc), mean S and N annual deposition rates, stand age, leaf type and habit, soil pH, the observed trends in LAI, S and N deposition, MAT, SPEI and the increase in CO_2 since the beginning of the C-flux measurements. To further test that the observed trends in C fluxes were dependent on the age of the stand, we calculated a surrogate of the state of maturity of the forests (Figure S2, Table S1) by dividing the mean stand age by the logging maturity tree age as described by Stokland *et al.* (2003) for average productivity classes. We checked for multicolinearity overseeing the variance inflation factor. The variance explained by each variable within these models was assessed using the proportional marginal variance decomposition (PMVD) metric from the relaimpo R package (Grömping, 2006).

The temporal contribution of each variable to the observed trends in GPP, Re, NEP and LAI was assessed using GLMMs and multi-model inference. Using the site as the random effect (or the pixel for the regional analysis) and an ARMA (p = 1, q = 0) autocorrelation structure, we fitted the saturated models as: response (annual anomalies) ~ (mean S deposition + S anomalies + CO_2) + (mean N deposition + N anomalies + CO_2) + (MATc + MAT anomalies + CO_2) + (MAPc + SPEI + CO_2) + mean S deposition x mean N deposition + MATc x MAPc, where variables between brackets where those for which we tested for interactions up to the second order. When including the interactions between the annual mean and the anomalies (e.g. MATc x MAT anomaly), we are including a changing effect of increasing or decreasing the anomalies depending on the mean for the site (e.g., increasing temperature may have a positive effect in cold climates but a negative effect in warmer climates). In C flux models, we used LAI as a surrogate for forest growth, which implies that the reported effects of increasing CO_2 concentrations are disconnected from any changes in LAI.

Using the model-averaging method [MuMIn R package] we used the saturated models for GPP, Re, NEP and LAI to construct an average model from the best models nested into the saturated models. Model averaging is a procedure based on multi-model inference techniques that computes an average model from the estimates of the best models predicting the data and weighting the relative importance of every model according to the difference of the second-order Akaike Information Criterion (AICc) between each model and the best model (with the lowest AICc) (Burnham & Anderson, 2004). Average models were calculated using those models differing by less than four AICc units (in comparison with the best model) and fitted using restricted maximum likelihood. Model residuals met the assumptions required in all analyses.

We then used the average models to predict the change of the response variables during the study period (1995–2011). We first extracted the observed trend (mean ± s.e.m) in our data using GLMMs. We then calculated the trend predicted by the average model and the trends predicted by the model, maintaining the predictors constant one at a time (e.g., S deposition anomalies are held constant while all other predictors change according to the observations). The difference between the predictions for the whole model and when one variable was controlled was the contribution of that predictor variable to the change in the response variable. The difference between all individual contributions and the observed trend were considered to be unknown contributions. Finally, we calculated the average NEP, GPP and Re sensitivities to predictor changes dividing the temporal contributions by the trends of the predictor variables. All errors were calculated using the error-propagation method.

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Block 2

Reproductive productivity and masting of forests

Chapter 5

The role of nutrients, productivity, and climate in determining tree fruit production in European forests

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Abstract

Fruit production and the amount of photosynthates allocated to reproduction are poorly quantified components of net primary production (NPP) in forest ecosystems. The controls of the spatial and specific variability of fruit NPP and its temporal behaviour are also poorly known for forests. To fill these gaps, we characterised fruit production and its temporal behaviour for several tree species and resolved the effects of gross primary production (GPP), climate, foliar nutrient concentrations, and atmospheric deposition of nitrogen (N) and sulphur (S). We used data for litterfall and foliar nutrient concentration from 126 ICP (International Co-operative Programme) forests distributed throughout Europe. We also used climatic data from the WorldClim database and EMEP (European Monitoring and Evaluation Programme) maps for N and S deposition. GPP was estimated for each forest using a regression model based on data from a global forest database. Mean fruit NPP ranged from approximately 10 to 40 g C m^{-2} y^{-1} and accounted for 0.5-3% of the GPP, with slight differences amongst species. Forests with higher GPPs and larger basal areas also produced larger fruit crops. Foliar zinc (Zn) and phosphorus (P) concentrations were associated positively with fruit crop size and negatively with its temporal variability. Foliar N concentration, C:P ratio, and the variability of annual precipitation were associated with higher variability of fruit production, and the N:P ratio was negatively correlated with fruit NPP variability. Fruit NPP and the percentage of GPP allocated to reproduction were similar amongst the species in the European forests. Foliar concentrations of N, P, and Zn and foliar nutrient stoichiometries determined the percentage of photosynthates allocated to reproduction and determined fruit NPP and its temporal behaviour in European forests.

1. Introduction

Fruit or seed production and its interannual variation (i.e. temporal behaviour) is an important life history trait in plants because it enables them to transmit their genes to the next generation (Fenner & Thompson, 2005). Fruit production in key species in an ecosystem can have significant effects on the entire community; fruit production regulates the populations of consumers (Espelta *et al.*, 2008) by providing intermittent resource pulses (Yang *et al.*, 2008), potentially producing cascading effects on food webs (Ostfeld & Keesing, 2000) that can even affect humans by the dispersion of illnesses such as lyme disease (Ostfeld, 1997). Fruit net primary production (NPP), however, is a poorly quantified component of total NPP.

Reproductive behaviour in some plant species (e.g. *masting* behaviour, the synchronous but highly variable production of seeds (Kelly & Sork, 2002)) is intriguing, so the production of fruit has received much attention from a wide range of ecologists. Most of the studies focusing on fruit or seed production, though, have quantified the number of fruits produced per tree (Sork *et al.*, 1993; Espelta *et al.*, 2008; Crone *et al.*, 2011; Fernández-Martínez *et al.*, 2015a), and few have provided data in units of mass produced per area (Campioli *et al.*, 2010; Herbst *et al.*, 2015; Pérez-Ramos *et al.*, 2015), which is essential for elucidating the contribution of reproduction to ecosystemic NPP. This neglect has been an important drawback for correctly estimating fruit NPP in studies of synthesis. The percentage of photosynthesised carbon (gross primary production, GPP) allocated to reproduction is also consequently mostly unknown, representing another drawback to the correct estimation of the carbon balance of forest ecosystems, because the mean residence time of carbon is very dependent on the compartment to which carbon is allocated (Fernández-Martínez *et al.*, 2015b). Providing measures of fruit NPP and the percentage of GPP allocated to reproduction would be of great interest to better close the carbon balance of ecosystems, especially in forests.

On the other hand, why some species or populations produce larger fruit crops or have more temporally variable fruit production than others is still not well understood. Most theories have mainly focused on the ultimate causes for these particular life-history traits (Silvertown, 1980; Kelly, 1994), but the proximate causes of the spatial variability in fruit production and its temporal behaviour have rarely been explored. Some authors have suggested that the size of the fruit crop from trees subjected to poor growing conditions (e.g. extreme or highly variable climates or poor soil properties such as low nutrient availability) should be more interannually variable (Kelly & Sork, 2002) because of the benefits of the *economy of scale* (Norton & Kelly, 1988; Smith *et al.*, 1990). Little empirical evidence, however, has yet been found suggesting that high interannual variability in fruit production is associated with poor growing conditions, such as drought (Fernández-Martínez *et al.*, 2012) or nutrient limitations, and the proximate causes for this association remain mostly unknown.

Endogenous factors such as stand age, basal area, or leaf habit and exogenous factors such as climate, nutrient availability, and anthropogenic impacts have been demonstrated to largely control NPP and GPP allocation to different NPP fractions at the global scale (Vicca *et al.*, 2012; Campioli *et al.*, 2015), even though different components of the NPP (e.g. foliage, stems, or roots) may be controlled by different factors (Fernández-Martínez *et al.*, 2014a). These endogenous and exogenous factors may thus also have an effect on fruit NPP.

Nutrient availability, in particular, may play an important role in determining fruit NPP and its temporal behaviour, because fruits and seeds are nutrient-rich (mostly in nitrogen [N], phosphorus [P], and potassium [K]) in comparison to vegetative tissues (Reekie & Bazzaz, 1987). Fruit production may thus have stronger relationships than vegetative tissues with nutrient availability (Sala *et al.*, 2012). N, for example, is the primary limiting nutrient for vegetative growth (Elser *et al.*, 2007; LeBauer & Treseder, 2008) and has also been positively correlated with investment in reproduction (Han *et al.*, 2013; Miyazaki *et al.*, 2014). N deposition has been suggested to increase forest productivity (Luyssaert *et al.*, 2010), but when combined with sulphur (S) deposition, the acidification of soils can have a negative impact on productivity and soil processes (Oulehle *et al.*, 2011; Büntgen *et al.*, 2013). Plants growing in P-deficient environments may also have a lower investment in sexual reproduction than those living in P-rich environments (Fujita *et al.*, 2014). Other macro- and micronutrients, often rarely studied in wild plants, may clearly also play an important role in determining fruit production and its temporal behaviour (Swietlik, 2002).

Elemental concentrations are important, but the ratios between the various elements are also important. The foliar N:P ratio can be an essential indicator of plant nutritional status and vegetative (Güsewell, 2004; McGroddy *et al.*, 2004; Sardans *et al.*, 2012, 2015) and reproductive (Fujita *et al.*, 2014) production. For example, species with low foliar N:P ratios tend to be fast-growing and more competitive for soil resources than species with higher N:P ratios (Willby *et al.*, 2001; Elser *et al.*, 2003; Peñuelas *et al.*, 2013). Other studies have suggested that, within the same species, flowering plants may have lower N:P ratios than non-flowering plants (Eckstein & Karlsson, 1997; Güsewell, 2004). Other elemental stoichiometries, such as C:N or C:P ratios, may also indicate limitations of soil nutrients (Fernández-Martínez *et al.*, 2014b) and may therefore also be associated with reproductive behaviour in plants.

The general aim of this study was to characterise fruit production and its temporal behaviour for several European tree species and to distinguish species-specific variability from the effects of productivity, foliar nutrient concentrations, climate, and atmospheric deposition of N and S. We specifically aimed i) to estimate average fruit NPP and the percentage of GPP (hereafter GPP%) allocated to fruit production of various tree species distributed across Europe, ii) to assess the temporal variation of the reproductive behaviour of these species by calculating the coefficient of variation (CV), the consecutive disparity index (D), and the temporal autocorrelation for lag 1 (AR1: the correlation between fruit production from years *y* and *y-1*), and iii) to identify the possible determinants of spatial and species-specific variability of fruit production and its temporal behaviour in relation to GPP, foliar nutrient (C, N, P, K, S, Fe, Mg, Ca, Zn, Mn, and Cu) concentrations and C:N:P (C:N, N:P, and C:P ratios) stoichiometries, climate (mean annual temperature and precipitation and their interannual variabilities), and atmospheric deposition of N and S.

2. Materials and methods

2.1. Data collection

2.1.1. Data for litterfall and foliar nutrient concentrations

We downloaded data for litterfall and foliar nutrient concentrations from the ICP Forests database (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forest, operated under the UNECE Convention on Long-range Transboundary Air Pollution, http://icp-forest.net/), containing data for fruit production for several forest species in Europe. Fruit and foliar litterfall for each site were summed over entire years, and foliar nutrient concentrations were averaged per plot. Average foliar C:N:P stoichiometries per site were calculated on a mass basis. The original data for litterfall were available in units of g of dry weight m^{-2} y^{-1}, so we used carbon-concentration data (provided by the same database) to calculate litterfall in units of g C m^{-2} y^{-1}. We also used site information such as mean plot diameter to calculate basal area. Of the 210 forests originally available, we used only 126 forests with time series with four or more years of data (Figure S5.1). We also excluded *Ceratonia siliqua* and *Larix decidua* because of the scarcity of replicates (two and one forest, respectively). We provided summary values for *Pinus nigra* but did not include this species in the statistical models, because only three replicates were available. Only 113 forests were finally used to fit the statistical models due to missing data for some of the predictor variables.

2.1.2. Climatic data

We extracted data for mean annual temperature and precipitation (MAT and MAP, respectively) for our forests from the WorldClim database (Hijmans *et al.*, 2005). This database provides climatic data with a high spatial resolution (30 arc seconds, ca. 1 km at the equator) and contains robust mean monthly climatic data derived from lengthy time series (1950-2000).

2.1.3. GPP data and the calculation of the fruit NPP-to-GPP percentage

We used a global forest database (Luyssaert *et al.*, 2007; Fernández-Martínez *et al.*, 2014b) to construct a model for estimating GPP from foliar NPP, leaf type, MAT, and MAP. We chose this set of predictors because these variables were also extracted for each of the forests used in this study, allowing the estimation of GPP for each site. The model, containing data for 84 forests, accounted for 74% of the variance in GPP. We crossvalidated the model using 75% of the data as a sampling set and the remaining 25% as the testing set and repeated this procedure 1000 times randomising both subsets. The crossvalidation indicated that the model performed acceptably well: average errors of the model predictions were lower than 12% for the testing set. The model and crossvalidation are summarised in the Supplementary Material (Figure S5.2 and Section 1: Estimating GPP). We calculated the percentage of GPP allocated to fruit NPP (fruit NPP-to-GPP percentage, hereafter %GPP) as: %GPP = 100 × fruit NPP × GPP^{-1}.

2.1.4. Atmospheric deposition data

We obtained data for atmospheric deposition from the EMEP gridded datasets and ICP Forests. Modelled N and S deposition data from the EMEP gridded maps were satisfactorily correlated with the ICP data for each forest (R=0.32 and 0.67, $P < 0.001$, for N and S deposition, respectively). We only used the EMEP data in our statistical models because the ICP data were incomplete for our database.

2.2. Data analyses

We calculated all the metrics of fruit production per plot from the annual values per site: fruit NPP, %GPP, maximum fruit NPP recorded in each forest, fruit NPP CV, fruit NPP D (Martín-Vide, 1986, Fernández-Martínez et al., *under review*), and fruit NPP AR1. CV and D were both used as measures of temporal variability. D was recently suggested to improve the assessment of temporal variability relative to the CV, especially in negatively autocorrelated time series (Fernández-Martínez *et al. under review*). AR1 indicates the temporal correlation between fruit crop sizes for years y-1 and y. Negative AR1 values in fruit production time series may be a sign of depletion of resources after large fruit crops (Sork *et al.*, 1993; Fernández-Martínez *et al.*, 2015a). These values were further averaged across sites per species to characterise the temporal behaviour of fruit NPP. We identified differences amongst species and leaf types using ANOVAs and Tukey's HSD tests for multiple comparisons.

We used a redundancy analysis (RDA) to characterise the relationships of the exogenous (MAT, MAP, and N and S atmospheric deposition) and endogenous (foliar nutrient concentrations, GPP, basal area, and leaf type) variables with the temporal behaviour of fruit production (mean fruit NPP, %GPP, and the CV, D, and AR1 of fruit NPP) using the "*vegan*" (Oksanen *et al.*, 2014) R package. RDA is a multivariate analysis, similar to the canonical correspondence analysis that correlates a set of predictor variables with a set of response variables. We excluded maximum fruit NPP from the redundancy analysis to simplify the model, because mean fruit NPP and maximum fruit NPP per plot were highly correlated ($R = 0.87$, $P < 0.001$). We constructed the model using a forwards-backwards stepwise model selection following the Akaike Information Criterion (AIC) starting from the null model. Significances of the constrained axes and the variables within the model were calculated using an ANOVA-like permutation test for RDA (*anova.cca* function in the *vegan* R library). Only significant variables remained in the model. We then performed a type I PERMANOVA to estimate the variance of the set of response variables explained by each predictor, entering them into the model in the order selected by the stepwise forwards procedure. A type II PERMANOVA (from the R package RVAideMemoire, (Hervé, 2015)), which is not sensitive to the order of the variables, was also used to assess the significance of the predictors. The PERMANOVAs used the species as the grouping factor within which to constrain the permutations (*strata* argument in *adonis* function, *vegan* R package).

As confirmatory analyses, we fitted generalised linear mixed models (GLMMs). Using the species as a random effect (to take into account, for example, the species effect on stoichiometry), we tested whether the fruit production metrics were correlated with productivity and forest characteristics, climate, foliar nutrient concentrations, and atmospheric deposition of N and S. The saturated model used GPP, basal area, N and S deposition, MAT, MAP, the CVs of MAT and MAP, foliar concentrations of C, N, P, K, S, Fe, Ca, Cu, Mg, and Zn, and foliar C:N, N:P, and C:P ratios as fixed effects. D was used for MAT and MAP instead of the CV for the models predicting D of fruit NPP. Variables were selected using the backwards-forwards stepwise method, starting from the saturated model. Once the most important variables were selected, we calculated the second-order interactions of the variables within the model and removed the non-significant terms. Models were fitted with the *lme* function of the R package "*nlme*" (Pinheiro *et al.*, 2013) using restricted maximum likelihood and a Gaussian distribution. We assessed the marginal (fixed factors) and conditional (fixed + random factors) variance explained by the model using the method proposed by Nakagawa & Schielzeth (2013) implemented in the MuMIn R package (Barton, 2015). We also used generalised additive models (GAMs) to explore the non-linearities between the response variables and the predictors using spline functions. Fruit NPP and D of fruit NPP were log-transformed to meet the assumption of normality and heteroscedasticity in the model residuals. All analyses were performed using R statistical software (R Core Team, 2015).

3. Results

3.1. *Species-specific variability in fruit production and its temporal behaviour*

Mean fruit production amongst the studied species ranged from (mean ± standard error) 6.1 ± 1.7 g C m^{-2} y^{-1} in *Pseudotsuga menziesii* to 40.6 ± 9.9 g C m^{-2} y^{-1} in *Pinus nigra* (Figure 5.1, Table S5.1). In fact, mean fruit NPP per year varied little amongst species and differed significantly only between the two above species and between *Pinus sylvestris* and *Quercus petraea* (Figure 5.1). Nonetheless, coniferous species had a higher average fruit NPP than broadleaved species when grouped (23.2 ± 2.1 and 15.0 ± 1.6 g C m^{-2} y^{-1}, respectively; ANOVA, $P = 0.003$). Similarly, the percentage of GPP invested in fruit NPP (%GPP) varied little amongst species (Figure 5.1) and, on average, was lower than 3% in all species (Table S5.1). The only differences amongst species were found between *P. sylvestris*, *P. menziesii*, and *Q. petraea*, with GPP% higher in *P. sylvestris* than the other two species. Leaf type, on the other hand, significantly affected %GPP, which was higher in coniferous than broadleaved forests (1.7 ± 0.2 and 1.1 ± 0.1%, respectively; ANOVA, $P = 0.004$). Maximum fruit NPP was similar across species and averaged 46.9 ± 3.1 g C m^{-2} y^{-1} (Figure 5.2, Table S5.1).

Temporal variability in fruit production differed substantially across species and between leaf types (Figure 5.3, Table S5.1). The deciduous species *Fagus sylvatica*, *Q. petraea*, and *Q. robur* had the highest temporal variabilities, with an average CV >1.3 and D >1.5 (Table S5.1). Both these indices of temporal variability indicated higher variability in fruit production in broadleaved than coniferous forests (1.38 ± 0.05 and 0.73 ± 0.05 for CV and 1.81 ± 0.09 and 0.85 ± 0.05 for D, respectively; $P < 0.001$). Also, the ANOVA indicated that the first autocorrelation coefficient (AR1) differed amongst species (Figure 5.3, Table S5.1). Tukey's HSD test, however, found significant differences only between *P. menziesii* and *F. sylvatica.* The former species had almost no autocorrelation, and the latter had a strong negative autocorrelation. Autocorrelation in fruit production generally tended to be negative, suggesting that years with higher (lower) fruit production tended to be followed by a year with lower (higher) fruit production.

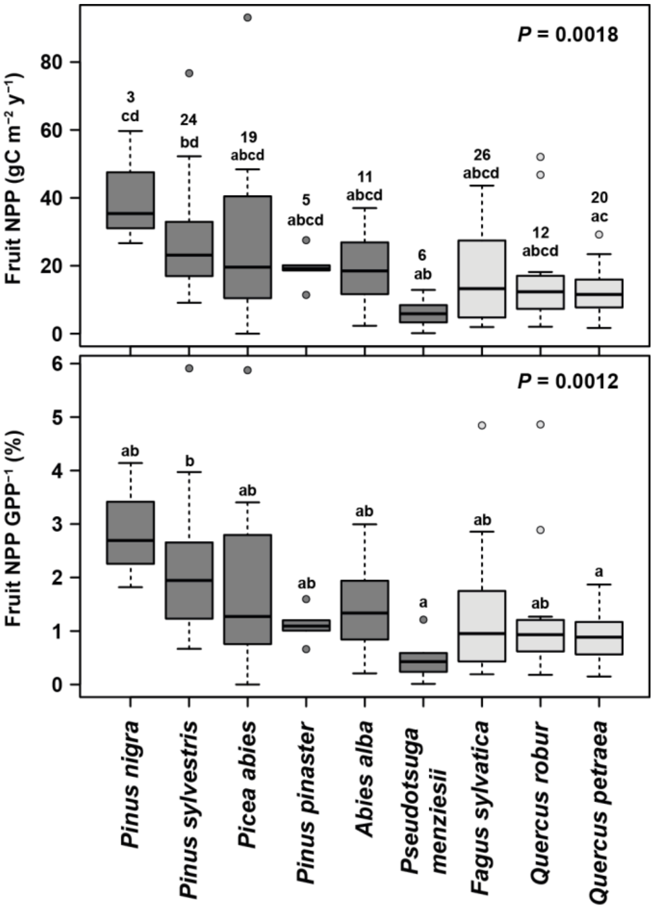

Figure 5.1: Box-and-whisker plots showing fruit NPP (upper panel) and fruit NPP-to-GPP percentage (lower panel) for nine European tree species. Dark grey boxes indicate coniferous species and light grey boxes indicate broadleaved species. The P values of the ANOVAs for differences amongst species are shown inside each panel. Different letters indicate significant differences ($P < 0.05$) amongst species (Tukey's HSD test for multiple comparisons). Numbers above the letters indicate the number of replicates. Average values are presented in Table S5.1.

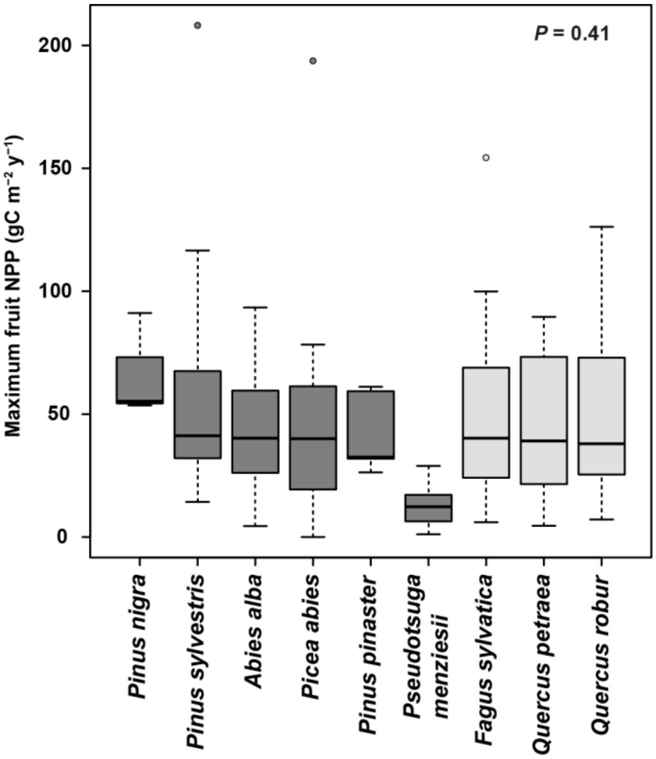

Figure 5.2: Box-and-whisker plot showing maximum fruit net primary production (NPP) (upper panel) for nine European tree species. Dark grey boxes indicate coniferous species and light grey boxes indicate broadleaved species. The *P* value of the ANOVA for differences amongst species is shown inside the plot. Average values are presented in Table S5.1.

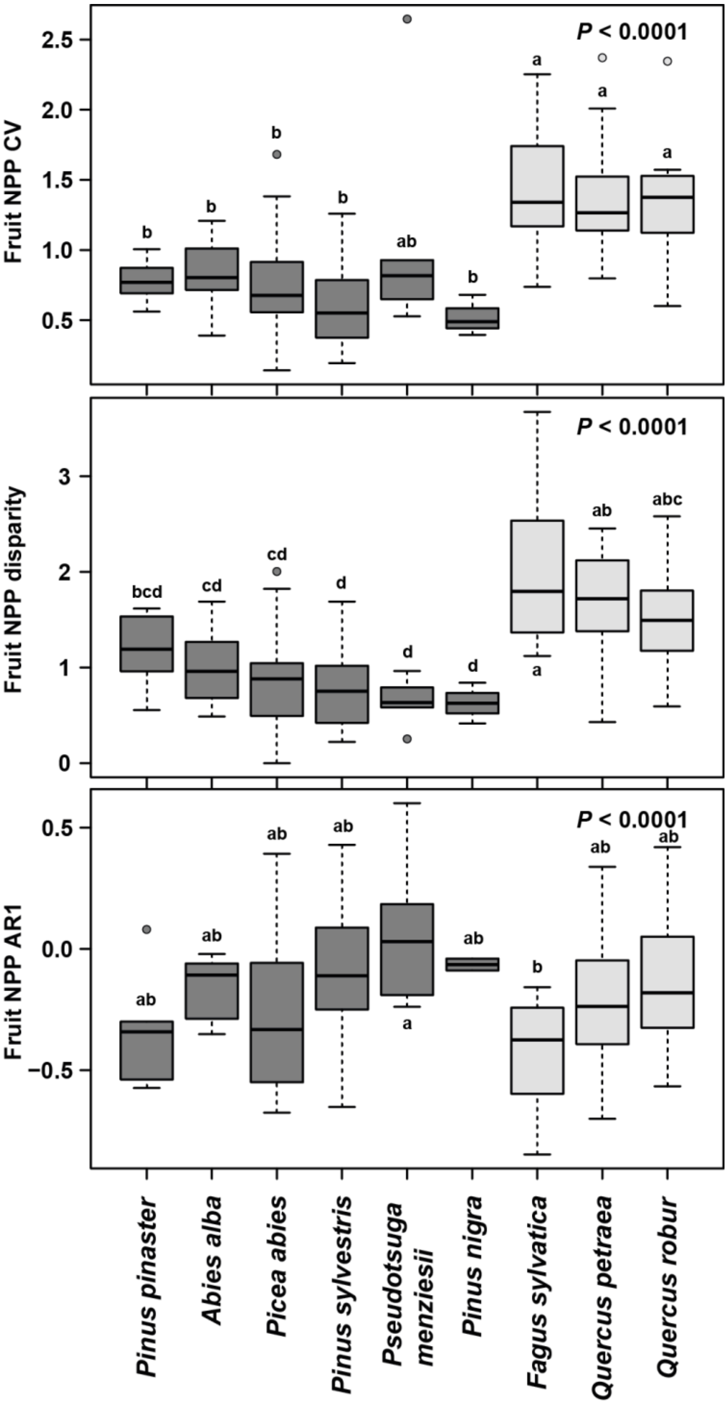

Figure 5.3: Box-and-whisker plots showing the coefficient of variation (CV, upper panel), disparity (D, middle panel), and autocorrelation coefficient for the first lag (AR1, lower panel) of fruit net primary production (NPP) for nine European tree species. Dark grey boxes indicate coniferous species and light grey boxes indicate broadleaved species. The *P* values of the ANOVAs for differences amongst species are shown inside each panel. Different letters indicate significant differences ($P < 0.05$) amongst species (Tukey's HSD test for multiple comparisons). Average values are presented in Table S5.1.

3.2. Determinants of fruit production and its temporal behaviour

The RDA indicated that GPP, MAP, and foliar nutrient concentrations of P, Zn, C, and S were the most important variables explaining the variability in fruit production (fruit NPP and %GPP) and its temporal behaviour (CV, D, and AR1 of fruit NPP) (Figure 5.4; Supplementary Information, section 2.1 Redundancy analysis). The first constrained axis, explaining 51% of the variability of the predictors, showed that GPP and foliar Zn and P concentrations were positively correlated with fruit NPP. Foliar S and C concentrations, however, were positively correlated with temporal variability in fruit NPP (characterised by CV and D). GPP% and AR1 were positively correlated with MAP. The second axis explained only 1% of the variance of the predictors and was not statistically significant. Type I and II PERMANOVAs confirmed the significance of the predictors selected by the RDA (Supplementary Information, section 2.2 PERMANOVA).

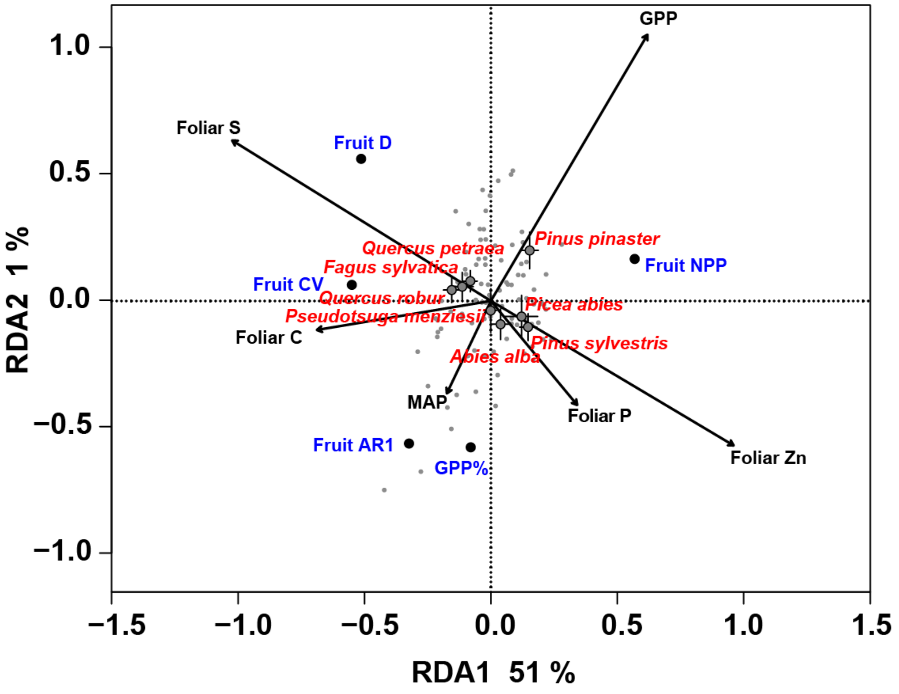

Figure 5.4: Plots showing the first two constrained axes of the redundancy analysis (RDA) using forest properties, climate, foliar nutrient concentrations, and atmospheric depositions of N and S as environmental variables (shown in black) and mean fruit net primary production (NPP), fruit NPP-to-GPP percentage, and the coefficient of variation (CV), disparity (D), and the autocorrelation coefficient for lag 1 (AR1) of fruit NPP as the matrix of species (shown in blue, see Material and Methods for further information about the methodology used to fit the model). The large grey dots (indicating mean ± SE) represent the "species" scores of the tree species in the analysis (red). The small grey dots indicate the environmental scores of the forests. GPP indicates gross

Confirmatory analyses using GLMMs indicated that GPP, foliar Zn and P concentrations (Figure 5.5), and mean basal area of the stand were positively associated with fruit NPP within and across species (Table 5.1). We also found a significantly positive interaction between foliar Zn and P concentrations (*P* = 0.009), indicating that the positive effect of Zn increased with increasing foliar P concentration (and vice versa) (Figure 5.6). Our analysis thus identified a synergic effect of foliar Zn and P concentrations, leading to higher fruit production when both nutrients occurred at high concentrations. The GLMM accounted for 63% of the variance in fruit NPP, with 43% explained by the fixed effects (GPP, Zn, P, and basal area) and 20% by differences amongst species.

Similar to fruit NPP, %GPP was higher in forests with high foliar Zn and P concentrations and large mean basal areas (Table 5.1). A significant interaction between foliar Zn and P concentrations (*P* = 0.002) was also significantly associated with %GPP. The model for %GPP explained 45% of the variance in %GPP, with 31% accounted for by the fixed effects and the remaining 14% by interspecific variability. Maximum recorded fruit NPP was associated positively with GPP and foliar P concentrations but negatively with foliar Mg concentrations (Table 5.1). The model accounted for 30% of the variance in maximum fruit NPP and showed no effect of interspecific variability.

The results for the temporal variability in fruit production differed depending on the index used to evaluate it, but both indices identified foliar Zn concentrations as an important driver of variability in fruit production (Table 5.1). The model predicting the CV of fruit production identified a negative interaction between MAT and foliar Zn concentrations, indicating that forests in warmer climates had a stronger negative relationship between foliar Zn concentrations and the CV of fruit production. Forests with higher foliar Zn concentrations, however, had a weaker positive relationship between MAT and the CV of fruit production. Our model further indicated that temporal variability in fruit production (CV) was lower in more productive forests (higher GPP) than less productive forests. We also found a positive relationship between foliar Cu concentrations and the CV of fruit production. Overall, the model explained 57% of the variance in the CV of fruit production, with 50% explained by the fixed effects and the remaining 7% by interspecific variability.

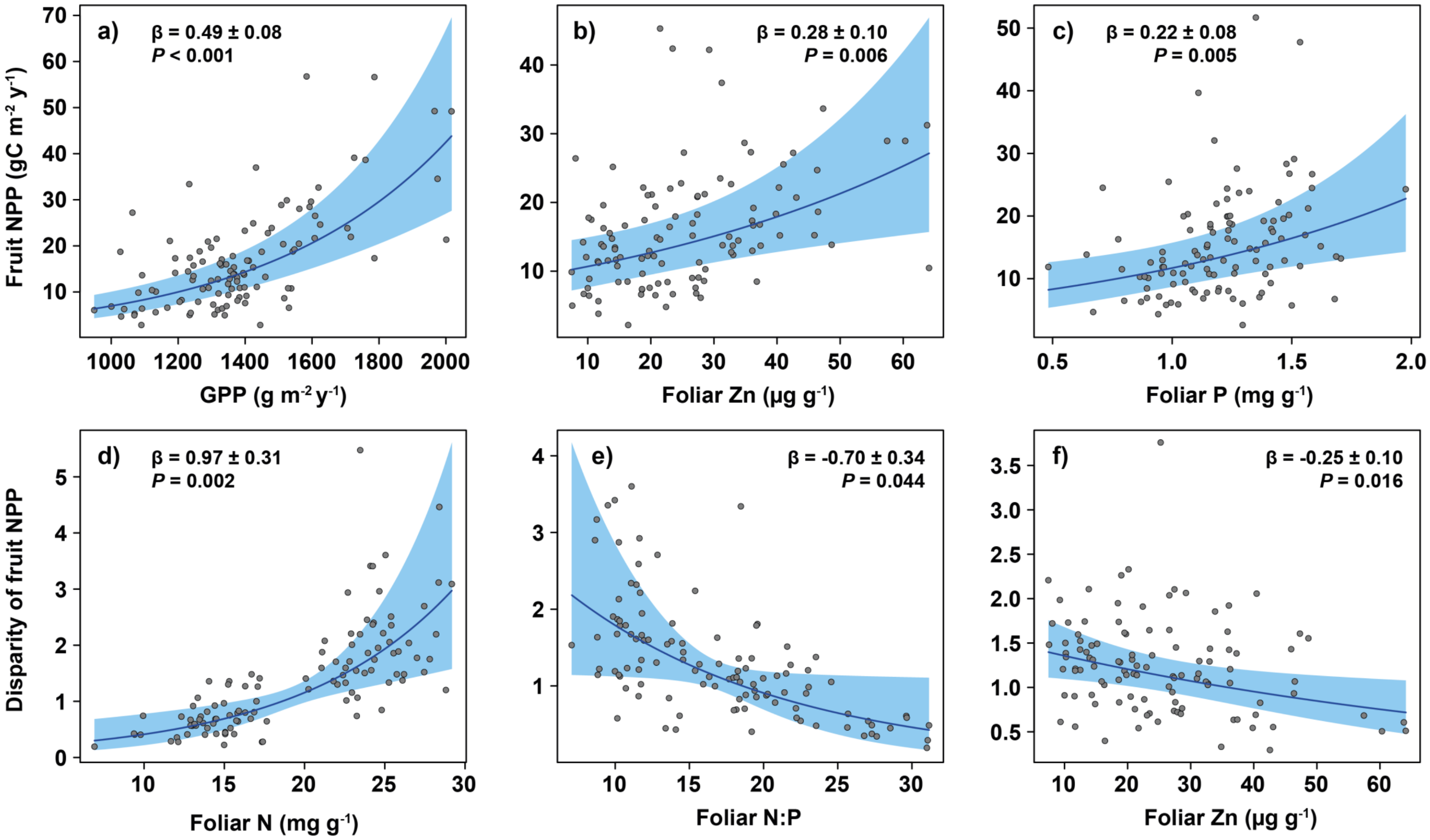

Figure 5.5: Partial residuals plots for the models of fruit net primary production (NPP) and the disparity of fruit NPP without interactions. Upper panels show the relationships of gross primary production (GPP) (panel a) and foliar Zn (panel b) and P (panel c) concentrations with fruit NPP. Lower panels show the relationships of foliar N (panel d) and Zn (panel f) concentrations and N:P ratio (panel e) with fruit NPP disparity. Beta weights (β ± SE) and their significance (*P*) within the model without interactions are shown in each panel. Light blue shading indicates 95% confidence bands.

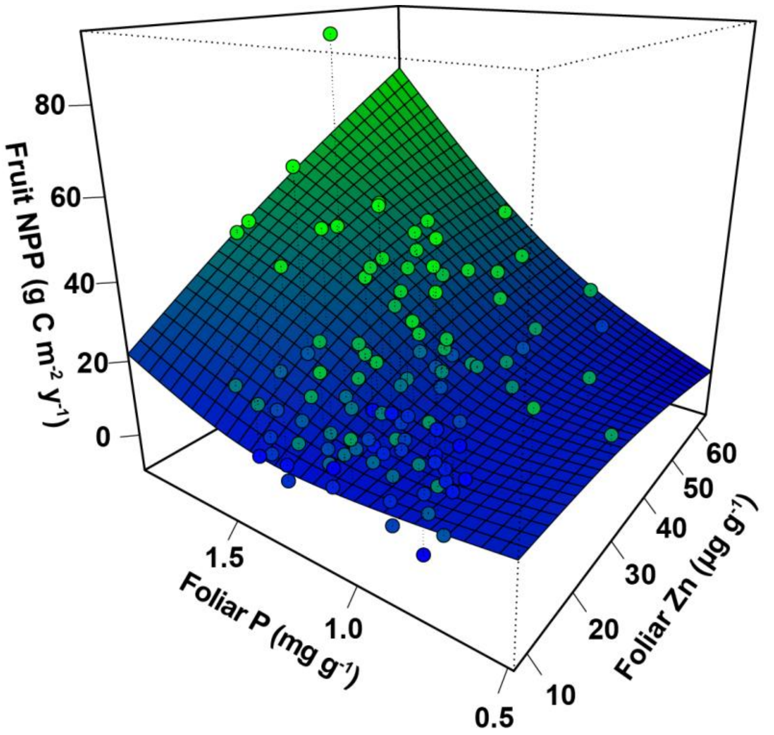

Figure 5.6: 3D plot showing the significant interaction between foliar P and Zn concentrations for predicting fruit net primary production (NPP). The response surface was calculated using a generalised additive model explaining 28% of the variance in fruit NPP.

The model predicting D of fruit production (Table 5.1) identified a positive interaction between foliar Zn concentration and the disparity of annual precipitation (MAP D), indicating that forests with more variable annual precipitation tended to have a weaker negative relationship between foliar Zn concentrations and D of fruit production and vice versa. Foliar Zn concentration (Figure 5.5) and MAP D, however, negatively and positively correlated, respectively, with D of fruit production when the interactions were removed from the model. The model (Table 5.1) identified a positive relationship between temporal variability in fruit production (D) and foliar N concentrations (Figure 5.5). Elemental stoichiometry also played a significant role in determining D of fruit production. Forests with higher foliar N:P ratios had lower temporal variability in fruit production, and high C:P ratios were associated with a high D of fruit production, especially at sites with high MAP D because of a positive interaction between the two. The model explained 49% of the variance in D of fruit production, with 35% of the variance attributed to the fixed factors and the remaining 14% to interspecific variability.

Finally, our model predicting temporal AR1 indicated that values of temporal autocorrelation were more negative for forests with high loads of N deposition than forests less exposed to N deposition. N deposition explained 7% of the variance in the temporal autocorrelation of fruit production, and species-specific variability explained 27%.

4. Discussion

4.1 Estimates of fruit NPP and forest C balance

Our results provide the first estimates of investment in NPP in fruit production for some of the most abundant tree species in European forests. Despite the high variability in the estimates, especially because of the differences in GPP and foliar nutrient concentrations (Table 5.1), average fruit NPP in our sample of European forests ranged from 10 to 40 g C m^{-2} y^{-1} and accounted for 0.5-3% of the GPP. These estimates of fruit NPP and GPP allocation are lower than those in previous studies. Herbst et al. (2015) reported mean fruit NPP estimates of 95 ± 37 and 73 ± 25 g C m^{-2} y^{-1} for managed and unmanaged stands of *F. sylvatica*, respectively. GPP allocation to fruit NPP averaged 6.7 ± 3.4 and 4.6 ± 2.0% in the managed and unmanaged stands respectively. In addition, GPP allocation to fruit NPP reached 23% in the managed stand during an exceptional mast year. Campioli et al., (2010) reported an average fruit NPP for *P. sylvestris* of 76 ± 8 g C m^{-2} y^{-1} and an average GPP allocation to fruiting of 6.0 ± 0.6%.

The differences between our results and previous reports of fruit NPP and allocation estimates may have different origins. Differences in the endogenous characteristics of the stands, such as GPP or foliar nutrient concentrations, are of major importance for fruiting NPP (Figure 5.5, Table 5.1). Moreover, exogenous factors such as the management of the stand can also influence carbon allocation to fruiting (Herbst *et al.*, 2015). The assessment of average values, however, is very dependent on the period of measurement due to the high irregularity of fruit production, especially in relatively short time series, as in our study. Different sampling methodologies could also be responsible for these differences in average fruit NPP.

Table 5.1: Summary of the mixed models predicting fruit net primary production (NPP), the percentage of gross primary production allocated to fruit NPP (%GPP), maximum fruit NPP, and the CV, D, and AR1 of fruit NPP. Values are beta weights ± SE. Variance explained for fixed factors corresponds to marginal R^2, and interspecific variance is the variance explained by the random effects. Ln indicates the response variable was transformed to the natural logarithm. Abbreviations: mean annual temperature (MAT), mean annual precipitation (MAP), coefficient of variation (CV), disparity (D), and autocorrelation coefficient at lag 1 (AR1). † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. All models included species as a random variable.

	Ln Fruit NPP	%GPP	Max Fruit NPP	CV	D	AR1
Endogenous variable						
GPP	0.5 ± 0.08 ***		0.47 ± 0.08 ***	-0.21 ± 0.08 **		
Basal area	0.16 ± 0.08 *	0.19 ± 0.09 *				
Foliar P	-0.2 ± 0.16	-0.21 ± 0.19 *	0.26 ± 0.08 **			
Foliar Zn	-0.61 ± 0.34 †	-0.95 ± 0.40		0.60 ± 0.47	-0.89 ± 0.29 **	
Foliar Mg			-0.17 ± 0.08 *			
Foliar Cu				0.37 ± 0.10 ***		
Foliar N					1.11 ± 0.30 ***	
Foliar N:P					-0.94 ± 0.34 **	
Foliar C:P					-0.27 ± 0.34	
Exogenous variable						
MAT				0.51 ± 0.19 **		
MAP D					-1.63 ± 0.53 **	
N deposition						-0.27 ± 0.10 *
Interaction						
MAT:Foliar Zn				-0.95 ± 0.47 *		
MAP D:Foliar C:P					1.45 ± 0.44 **	
MAP D:Foliar Zn					1.04 ± 0.45 *	
Foliar P:Foliar Zn	1.02 ± 0.38 **	1.42 ± 0.45 **				
Variance explained						
Fixed factors	0.43	0.31	0.30	0.50	0.35	0.07
Interspecific variability	0.20	0.14	0.00	0.07	0.14	0.17
Total	0.63	0.45	0.30	0.57	0.49	0.24

Fruit NPP is usually a relatively small component of the carbon balance of forest ecosystems, but its high interannual variability in masting species, such as *F. sylvatica* or *Quercus* sp., identifies fruit NPP as an important component to consider (Herbst *et al.*, 2015). Mean maximum fruit NPP was 2- to 3-fold higher than mean fruit NPP for all species (Table S5.1). %GPP can thus increase substantially in years with large fruit crops. Further analyses with longer time series are clearly needed to obtain more robust estimates of fruit production, but our results provide new insights on the fate of photosynthesised C in forest ecosystems.

4.2 The role of nutrients in fruit NPP and its temporal behaviour

Our forests were in the lower range of mean concentrations of foliar Zn (Table S5.2), indicating that some of the forests might be Zn deficient (Swietlik, 2002). Zn deficiency has been well studied in agricultural crops, but to the best of our knowledge, we are the first to report a potential role in forest ecosystems. Zn deficiency usually occurs in plants growing on alkaline soils because of the reduced bioavailability of Zn (Ma & Lindsay, 1990) but can also occur in acidified weathered soils (Alloway, 2009). Zn limitation can have a negative impact on plant vegetative growth and especially fruit yield (Swietlik, 2002) because of the roles Zn plays in several key metabolic processes such as protein synthesis (as a component of ribosomes, Prask and Plocke, 1971) or in metalloenzymes such as carbonic anhydrases (Dell & Wilson, 1985) involved in the conversion of carbon dioxide and water to bicarbonate and protons (photosynthesis and biomass production). Cakmak and Marschner (1988) found that Zn deficiency in *Gossypium hirsutum*, *Triticum aestivum*, *Lycopersicon esculentum*, and *Malus domestica* increased the exudation of K, amino acids, sugars, and phenolics from roots because of increased membrane permeability. Zn-deficient plants may thus have less photosynthates available for aboveground compartments (Vicca *et al.*, 2012; Fernández-Martínez *et al.*, 2014b, 2015b) and fruit production (Figures 5.5 and 5.6). These processes may also be responsible for increased temporal variability in seed production, because Zn-limited forests may not have enough reserves to regularly produce large fruit crops (Isagi *et al.*, 1997).

Forests with higher foliar P concentrations had higher mean fruit production and allocation to fruit production in our study, supporting the finding by Fujita *et al.* (2014). P had a strong effect, especially in forests with high foliar Zn concentrations (Table 5.1). This synergistic effect of foliar P and Zn concentrations could be a consequence of an induced limitation of one nutrient when the other is enriched, (Elser *et al.*, 2007) in agreement with von Liebig's law of the minimum (Von Liebig, 1840). The close association of P (Elser *et al.*, 2003) and Zn (Prask & Plocke, 1971) with ribosomes, and therefore with metabolism and biomass production, further supports a positive interaction between these elements. Maximum fruit NPP was higher in forests with high foliar P concentrations, and the foliar C:P ratio was positively associated with a high disparity in seed production. P, like Zn, may thus be a limiting factor for fruit production, increasing temporal variability when deficient. These results also agree with previous reports suggesting that higher foliar P concentrations produce larger fruit crops compared to those with lower concentrations (e.g. in olive trees (Erel, 2008) and amongst multiple plant species (Fujita *et al.*, 2014)) or, more generally, that P limitation constrains NPP in forests (Perkins, 2004; Plassard & Dell, 2010) and in freshwater, marine, and other terrestrial ecosystems (Elser *et al.*, 2007; Peñuelas *et al.*, 2013).

Trees with higher foliar N concentrations (mostly broadleaved deciduous species; Figure 5.5, Table 5.S5.2) did not have higher fruit NPP but had a higher temporal variability of fruit NPP than those with lower N concentrations (mostly coniferous species). N has been identified as the primary limiting nutrient for vegetative growth (Elser *et al.*, 2007; LeBauer & Treseder, 2008) and reproduction (Han *et al.*, 2013; Miyazaki *et al.*, 2014), but the lack of association between foliar N concentration and fruit NPP might be a consequence of other nutrients limiting production, such as Zn or P. Europe has high rates of N deposition, which may increase N availability for plants, indicated by the relatively high average values of foliar N concentrations in European forests (Table S5.2). The positive relationship between foliar N and fruit temporal variability, however, may indicate that forests with higher N reserves could be able to produce larger fruit crops when other factors are not limiting growth during a particular year (such as weather, other nutrients such as Zn or P, or a combination of both (Fernández-Martínez *et al.*, 2015a)). This reasoning is also consistent with the negative relationship between atmospheric N deposition and AR1 of fruit NPP and explains the negative relationship of N:P ratios, which are typical of fast-growing species (Willby *et al.*, 2001; Elser *et al.*, 2003) with fruiting temporal variability. Conversely, higher foliar C:P ratios in our study were associated with higher variability in fruit production, especially at sites with highly variable precipitation (Table 5.1). This finding is consistent with P limitation for fruit production: P-deficient forests may not be able to produce fruit as regularly as those with sufficient P availability.

Our results thus indicate that nutrient-rich forests produce more fruit, allocate a larger fraction of the GPP to fruit production, and produce fruit more regularly than those in nutrient-limited environments, supporting previous findings suggesting higher C allocation to aboveground NPP in nutrient-rich forests (Vicca *et al.*, 2012; Fernández-Martínez *et al.*, 2014b, 2015b) and supporting masting theory and research suggesting that masting behaviour is more pronounced in environments where resources are scarce (Kelly & Sork, 2002; Fernández-Martínez *et al.*, 2012). Nutrient availability may thus limit fruit NPP even more than vegetative NPP, because fruits are more nutrient-demanding than vegetative tissues (Reekie & Bazzaz, 1987). Foliar Zn and P concentrations were especially associated with higher fruit production.

4.3 Other determinants of fruit NPP and its temporal behaviour

Nutrient availability and stoichiometry played a very important role in determining fruit NPP and its temporal behaviour, but productivity and climate were also key factors. Higher photosynthesis (GPP) was associated with higher fruit NPP, higher maximum fruit NPP, and lower temporal variability (CV). This result is logical, because forests with larger GPP fluxes also have larger NPP fluxes (Fernández-Martínez *et al.*, 2014a). Whether all NPP components increase equally with increasing GPP, however, remains unresolved. In addition, higher GPP might reduce the CV of fruit production because: i) more photosynthates can be allocated as reserves for reproduction, allowing more regular fruit crops, or ii) a spurious relationship is introduced, due to the positive relationship between fruit NPP and GPP and the dependence of the CV on the mean (Fernández-Martínez *et al. under review*). This second hypothesis is supported by the lack of a relationship between D (which is less sensitive to the mean than the CV (Fernández-Martínez *et al. under review*)) of fruit production and GPP. A larger basal area, however, was also associated with higher fruit NPP and GPP%, perhaps due to the competitive advantage of large trees to acquire various resources (e.g. nutrients, water, and sunlight).

The positive association between MAT and the CV of fruit NPP was influenced by foliar Zn concentrations. Temperature and foliar Zn may have a negative interaction because warmer European sites (southern forests, Figure S5.1) are more often subjected to droughts and high temperatures, resulting in massive fruit abortion (Espelta *et al.*, 2008; Pérez-Ramos *et al.*, 2010; Fernández-Martínez *et al.*, 2012) and therefore more irregular fruit production. Forests with higher foliar Zn concentrations might be less prone to fruit abortion, which could reduce temporal variability in fruit production. Similarly, D of fruit production was positively associated with MAP D, especially in forests with high foliar C:P ratios (therefore mostly P-limited forests) and with high foliar Zn concentrations. The P-dependent relationship between temporal variability in precipitation and size of fruit crop supports the hypothesis that highly constrained trees have stronger masting behaviour (Kelly & Sork, 2002). The positive interaction between foliar Zn and D of precipitation could emerge as a result of the higher productivity in rainy years of Zn-rich compared to Zn-deficient forests. Precipitation and drought are amongst the most important factors driving fruit and secondary production (Ogaya & Peñuelas, 2007; Pérez-Ramos *et al.*, 2010; Garbulsky *et al.*, 2013). Mediterranean forests are especially subjected to high inter-annual variability in water availability and could thus exhibit higher temporal variability in fruit NPP. Our results thus indicate that nutrient availability has the potential to alter the close association between the variability of weather and the behavior of fruit production in European forest ecosystems (Fernández-Martínez *et al.*, 2015a).

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Chapter 6

The North Atlantic Oscillation synchronises fruit production in central European forests

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Abstract

Weather (temperature and precipitation), and its lagged effects, have been usually related to interannual variability and synchrony of fruit production for several tree species. These relationships have often been used to discuss hypotheses relating interannual variability in fruit crop size with tree resources or favourable pollination conditions, and synchrony in fruit production among sites with the Moran effect (the synchronization of biological processes among populations driven by weather variability) or the local availability of pollen. Climatic teleconnections, such as the North Atlantic Oscillation (NAO), representing *weather packages*, have been, however, barely related to fruit production despite they often predict ecological processes better than local weather. The aim of this study was to test whether seasonal NAO indices were useful in order to predict interannual variability and synchrony in fruit production using 76 forests of *Abies alba*, *Fagus sylvatica*, *Picea abies*, *Pseudotsuga menziesii*, *Quercus petraea* and *Quercus robur* distributed across central Europe. For all species studied, we found interannual variability in fruit production to be significantly related to the seasonal NAO indices and that had a most prominent importance than local weather variables in predicting it. The relationships emerged from these analyses pointed out to proximal causes as the main responsible for interannual variability in fruit crop size, supporting that tree resources and favourable pollination conditions are needed to produce large fruit crops. Synchrony in fruit production between forests was mainly controlled by weather and the geographical distance among sites. Also, for a given year, fruit production was less variable among sites during warm and dry springs (negative spring NAO phases). Our results pointed out the Moran effect as the most likely mechanism for synchronization of fruit production at large geographical scales. However, our results indicate the possibility that pollen availability plays a role in synchronizing fruit production at local scales. Our results highlight the influence of the NAO on fruit production patterns over central Europe.

1. Introduction

Fruit and seed production is an essential step in the life cycle of plants that allows individuals to transmit their genes to the next generation and to colonize new territories or remain where the progenitors are established. However, plant reproduction has important implications beyond the plant itself, especially if it concerns the key species of the community: production of fruits can alter the whole ecosystem by producing cascading effects through the food web (Elkinton *et al.*, 1996; Ostfeld & Keesing, 2000), even affecting the transmission of diseases such as the lyme disease to humans (Ostfeld, 1997). Therefore, different behaviours of fruit production would have different consequences for the ecosystem.

Two main hypotheses have tried to explain interannual variability in fruit production. On the one hand, the mechanistic *resource matching* hypothesis (Norton & Kelly, 1988; Sork *et al.*, 1993; Kelly & Sork, 2002) suggests that plants produce fruits as a direct response of the available resources (Table 1). On the other hand, the *pollination efficiency* hypothesis (Norton & Kelly, 1988; Smith *et al.*, 1990; Koenig & Ashley, 2003), states that synchronized and intermittent flowering increases pollination success in wind pollinated species because ensures a high percentage of pollination success during years of large flowering efforts (Table 1). Both hypotheses can be related to weather variability since weather can influence both, the acquisition of tree resources (e.g., through increased photosynthesis) and favourable environmental pollination conditions.

Similarly to interannual variability, synchrony in fruit production has also been mostly explained by two hypotheses. On the one hand, the Moran's theory (Moran, 1953; Ranta *et al.*, 1997), states that synchrony in fruit production is controlled by synchrony in weather conditions, which prompt the trees to divert resources into reproduction or, mechanistically, producing more fruits when weather is favourable for acquiring more resources (Table 1). Since weather conditions can also be spatially synchronous (Koenig, 2002), the Moran effect has also been hypothesised to be a potential synchronizer of fruit production over large geographic areas (Koenig & Knops, 2013). On the other hand, the *pollen coupling* hypothesis (Satake & Iwasa, 2002) suggests that the available pollen from neighbour trees can generate synchrony in fruit production among individuals by interacting with available resources for trees (Table 1, i.e., a large maturation of female flowers after a massive pollination event (when enough pollen is available) would deplete the resources of all trees at a time, leading to the synchronization of fruit production within the population).

Because of the importance of weather variability for plant productivity, most of the studies that have tried to test these hypotheses have based their conclusions on the relationship between interannual variability and synchrony in fruit production with weather conditions (Sork *et al.*, 1993; Fernández-Martínez *et al.*, 2012; Koenig & Knops, 2013). Recent publications have highlighted the possible role of temperature as a weather cue (Kelly *et al.*, 2013; Kon & Saito, 2015) although other authors have suggested temperature to likely act as proximal causes when predicting fruit crop size (Pearse *et al.*, 2014). In any case, temperature variability would also explain synchrony in fruit production since changes in temperature occur at wide geographical scales (Koenig, 2002). However, other weather variables (e.g., precipitation or water stress) could also be used as weather predictors (Sork *et al.*, 1993; García-Mozo *et al.*, 2007; Espelta *et al.*, 2008; Fernández-Martínez *et al.*, 2012) to be related to interannual variability and synchrony in fruit production.

Weather at continental scales is mostly driven by general atmospheric circulation patterns. Climatic teleconnections have been demonstrated to have a strong influence on weather over very large areas. El Niño/Southern Oscillation is known to affect the weather of the whole planet (Grove, 1998; NOAA, 2012) and the North Atlantic Oscillation (NAO, the dipole connecting the Icelandic low with the Azores high) is known to strongly affect the Atlantic basins of Europe and North America (Hurrell *et al.*, 2002, 2003). Accordingly, ecosystems have also been suggested to be affected by teleconnections (Straile, 2002; Menzel *et al.*, 2005; Martínez-Jauregui *et al.*, 2009; Hódar *et al.*, 2011) and some authors have suggested that teleconnection indices often predict ecological processes better than local weather (Ottersen *et al.*, 2001; Stenseth *et al.*, 2003; Hallett *et al.*, 2004), for being an aggregate of weather conditions over large spatial scales. Accordingly, the NAO index may act as an excellent weather cue. However, up to date, the role of climatic teleconnections has been rarely explored in studies focused on fruit production (but see: Wright et al. 1999). Additionally, most of the published literature exploring the effects of the NAO on ecosystems has focused on the winter values of the index (Ottersen *et al.*, 2001). This can be a limitation of the approach, since different biological processes might be influenced by different seasonal weather.

Fruit production in some species has been shown to be strongly correlated to weather from the warm seasons (Sork *et al.*, 1993; Fernández-Martínez *et al.*, 2012, 2015; Kelly *et al.*, 2013; Pearse *et al.*, 2014) for which the winter NAO may have little influence. Tree species with contrasting leaf habit (evergreens and deciduous) may be sensitive to weather conditions during different seasons. Winter-deciduous species have to accumulate enough resources prior to winter for leaf unfolding and flowering, so it is likely that autumn weather has some influence on next year's productivity. On the other hand, since evergreen species preserve leaves during winter it is possible that winter weather variability play a role determining plant productivity during the next year. Exploring the effects of the NAO index for other seasons may also provide interesting results and even improve the prediction capacity of the models.

In our study, we aim to test whether seasonal NAO indices can predict interannual variability in fruit production by constructing statistical models including local weather (temperature and precipitation) variables and NAO indices for previous autumn, winter, spring and summer seasons, using 76 forests of *Abies alba*, *Fagus sylvatica*, *Picea abies*, *Pseudotsuga menziesii*, *Quercus petraea* and *Quercus robur* distributed across Europe. We also aim to explore which environmental variables can explain synchrony in fruit production among forests within species. Finally, we aim to discuss the possible contribution of the different hypotheses trying to explain interannual variability and synchrony in fruit production with the results provided by the statistical models.

Table 1: Summary of the hypotheses discussed in this study for interannual variability and synchrony in fruit production. Because this analysis is focused on weather relationships with interannual variability and synchrony in fruit production, only indirect evidences through weather relationships supporting or rejecting the hypotheses are presented.

		Indirect evidences	
Hypothesis	**Summary**	**Supports**	**Rejects**
a) Interannual variability			
Resource matching	Plants produce fruits as a direct response of the available resources for plants.	Favourable weather for tree productivity is positively correlated with fruit production.	No weather correlation or weather correlation cannot be associated to higher tree productivity.
Pollination efficiency	Synchronized and intermittent flowering increases pollination success in wind pollinated species.	Favourable weather for pollination (e.g., warm and dry springs) is positively correlated to fruit production.	Favourable weather for pollination is negatively or not correlated to fruit production.
b) Synchrony			
The Moran effect	Synchrony in fruit production is driven by synchrony in weather conditions.	Fruit production among sites is correlated with the same weather conditions. Non-hybridizing species are also synchronized.	Fruit production among sites is correlated with different weather conditions or no significant synchrony in fruit production is found among sites.
Pollen coupling	Pollen availability from neighbour trees can generate synchrony in fruit production among individuals by interacting with available resources.	Favourable weather for pollination is positively correlated to fruit production. Synchrony happens within species at the local scale.	Unfavourable weather for pollination is correlated to fruit production. Non-hybridizing species are synchronized.

2. Materials and methods

2.1. Data collection

2.1.1. Litterfall and foliar nutrient concentration data

We downloaded litterfall data from the ICP forests database (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forest, operated under the UNECE Convention on Long-range Transboundary Air Pollution, http://icp-forest.net/), containing fruit production data for several forest species in Europe covering the period 2002 - 2010. Fruit litterfall was summarized per plot and year. Since original data on litterfall was available in units of g of dry weight m^{-2} y^{-1} we used fruit carbon content data (provided by the same database) to calculate litterfall in units of g C m^{-2} y^{-1} (as fruit Net Primary Production [NPP]). From the 210 forests originally available, we only used 76 monospecific forests, distributed over France, Germany and Luxemburg, containing time series with 5 or more years of data belonging to *Abies alba*, *Fagus sylvatica*, *Picea abies*, *Pseudotsuga menziesii*, *Quercus petraea* and *Quercus robur* (Figure 1).

2.1.2. Weather data

We extracted weather time series for our forests from the interpolated meteorological data of the MARS unit AGRI4CAST/JRC (http://agri4cast.jrc.ec.europa.eu/), with a resolution of 0.25 x 0.25 degrees (latitude, longitude). This database provided monthly values of mean temperature and precipitation sum from 2001 to 2010. We also downloaded the NAO index time series on a daily basis, covering the period comprised between the 1st of January of 1950 to the 31th of December of 2014, from the Climate Prediction Center of National Weather Service (NOAA), http://www.cpc.ncep.noaa.gov/. We then calculated seasonal NAO indices, temperatures and precipitations of winter, spring, summer and autumn. We calculated winter values as the average (for temperature and NAO) or the sum (for precipitation) of January, February and March; spring comprised the months of April, May and June; summer comprised the months of July, August and

September and autumn comprised the months of October, November and December. Seasonal NAO indices were standardized for the period 1950 – 2014.

2.2. Data analyses

2.2.1. Interannual variability of fruit production

A summary of the analyses performed in this study can be found in Table 2. To evaluate the influence of seasonal NAO indices on seasonal local weather (temperature and precipitations) we fitted generalised linear mixed models (GLMM) with the "*nlme*" R package (Pinheiro *et al.*, 2013), using restricted maximum likelihood (REML) and a Gaussian distribution, where the site was the random factor and the different seasonal NAO indices were related to seasonal temperatures and precipitations.

To assess whether seasonal NAO indices were correlated to fruit production, we performed spearman correlations per site between seasonal NAO indices and fruit production time series. Then, we calculated the average correlations between fruit production and seasonal NAO indices per species. Next we fitted generalised linear mixed models (GLMM), using restricted maximum likelihood (REML) and a Gaussian distribution, where the site was the random factor. To take into account the effect of the previous fruit crop, models also included an autoregressive term for the lag 1 (ARMA [1,0], crop year i ~ crop year i-1). Saturated models (models including all possible predictors) predicting interannual variability in fruit production included temperature, precipitation and NAO indices for the autumn, winter, spring and summer seasons previous to fruit ripening (e.g., fruit crop year i ~ autumn temperature year i-1 + winter temperature year i +…). Variable selection for each model was achieved using the "*dredge*" function in MuMin R pacakage (Barton, 2015) by means of the best subset model selection, using the Bayesian Information Criterion (BIC) as the measure of model adjustment (the best model is the model with the lowest BIC). We also calculated the variance explained by the fixed factors (marginal variance, R^2_m) and by the whole model (conditional variance, R^2_c) for the final models using the methodology proposed by (Nakagawa & Schielzeth, 2013) implemented also in the MuMIn R package (Barton, 2015) by the function "*r.squaredGLMM*". The difference between the marginal and the conditional variance explained was the variance explained by the random factors. To assess the importance of the predictors within the models, we calculated the ΔBIC for each of them, as the difference between the BIC of the final model and the BIC of the model without the predictor of interest. The higher the ΔBIC value, the larger the importance of the predictor within the model. To meet the assumption of normality and heteroscedasticity in model residuals fruit NPP was log-transformed. In these analyses, we excluded sites with less than 5 years of data. We also used mixed models with random slopes to test the effect of the seasonal NAO indices on temperature and precipitation, using the site as the random factor.

Relationships between fruit production and weather variables involved either with pollination success (supporting the *pollination efficiency* hypothesis) or with greater tree productivity (supporting the *resource matching* hypothesis), would indicate an effect of weather on fruit production through proximal causes. Instead, if the relationship between weather and fruit production cannot be related to well-established physiological responses (e.g., warm and wet conditions normally increase tree productivity), results may be supporting the role of weather as a cue for fruit production.

2.2.2. Synchrony of fruit production among forests

We first tested whether, for a given year, higher variability in weather conditions among sites was associated to higher variability in fruit production. To this end, for each of the years with records for more than 5 forests per species, we calculated the annual coefficient of variation (CV = standard deviation · mean^{-1}) among sites of seasonal (winter, spring, summer and autumn) temperature, precipitation and annual fruit production for each species. Then, we used generalized linear mixed models (GLMM), using the species as the random factor, to check for the significance of the association between annual fruit production CV and weather CV among sites and the seasonal NAO indices. Positive associations between annual fruit and weather CV would further support the Moran effect hypothesis, and no association would indicate that other mechanisms, such as the pollen coupling, might be involved in synchronizing fruit production.

Then we calculated the degree of synchrony in fruit production among all sites using Spearman's correlations (ρ) for all those sites sharing data for at least 5 years. Next, we calculated the mean correlation of fruit production per species and among species. Then we tested which were the main responsible controls of synchrony in fruit production among forests within species. To that end, we first calculated synchrony (ρ) for weather (seasonal temperatures and precipitations) among forests of the same species and, using linear models, we tested for the relationship of synchrony in weather and synchrony in fruit production with geographical distance. The Moran effect would be supported if the slope between distance and synchrony in seasonal temperature or precipitation among sites is as high as or higher than the slope between distance and synchrony in fruit production among sites (similar slopes using a t-test, $P>0.05$). If slopes differ or there is no spatial synchrony in fruit production among forests, results would then provide indirect evidences supporting the *pollen coupling* hypothesis because it could indicate that synchrony happens at more local scales (Table 1).

Afterwards, we constructed linear models in which the response variable was the synchrony (ρ) of fruit production between two forests, and the predictor variables were the geographical distance between sites and the correlation between winter, spring, summer and autumn temperatures and precipitations of the two forests. Next, we performed the model selection using the "*dredge*" function in MuMin R pacakage (Barton, 2015) by means of the best subset model selection, using the Bayesian Information Criterion (BIC) as the measure of model adjustment. In that case, the best model was assumed to be the one with the lowest BIC having all variables significant at the 0.05 level and with no negative coefficients in weather variables (since negative associations between synchrony in fruit production and in weather variables would be nonsensical). The percentage of variance explained by the predictors was assessed using the proportional marginal variance decomposition metric "*pmvd*" from the *relaimpo* R package (Grömping, 2007).

Similar to results of interannual variability, significant relationships between synchrony in fruit production and synchrony in weather variables from periods potentially related to tree productivity would agree with the Moran effect taking place as a result of proximal causes of weather on fruit production. On the other hand, if weather variables related to synchrony in fruit production can be related to spring conditions, in which pollination takes place, the *pollen coupling* hypothesis would be supported (Table 1). Instead, if weather predictors of fruit synchrony cannot be associated to the pollination period nor to greater tree productivity, results would agree with the Moran effect taking place because of weather acting as a cue for trees.

We used the *visreg* R package (Breheny & Burchett, 2015) to visualise the regression models using partial plots. All data treatment and analyses were conducted using R (R Core Team, 2015).

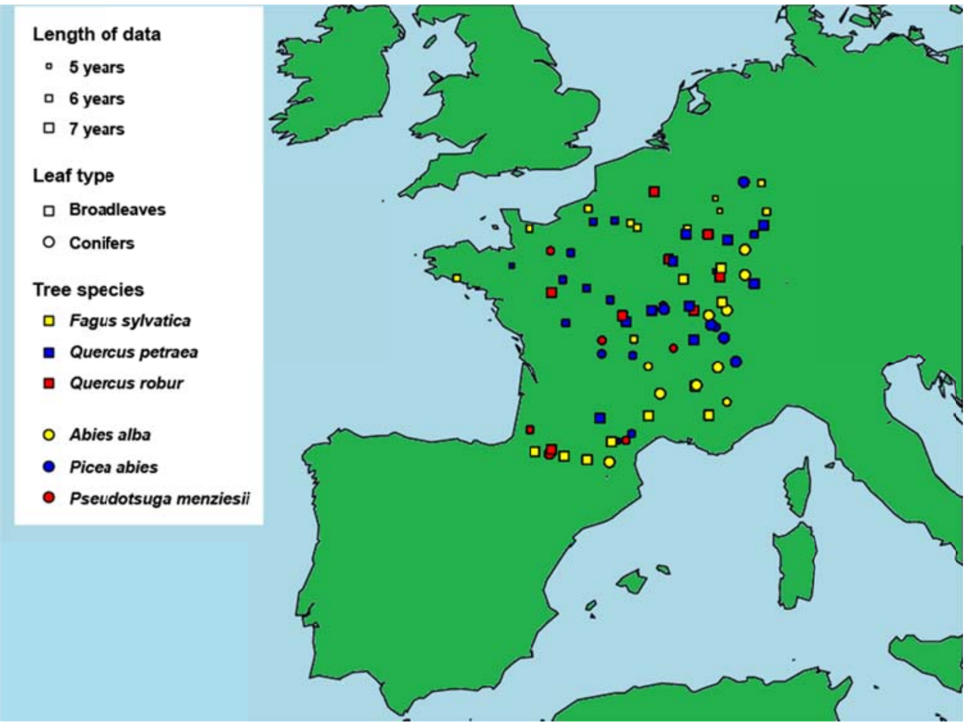

Figure 1: Map showing the sites used in this study. All sites were located through France, Germany and Luxembourg.

Table 2: Summary of the specific aims of the study and the statistical tests performed.

Questions	Analyses
a) Weather variability	
Do seasonal NAO indices correlate with seasonal weather in our sites?	Generalized linear mixed models (with site as random factor) relating seasonal temperature and precipitation to seasonal NAO indices.
b) Interannual variability in fruit production	
Do seasonal NAO indices correlate with fruit production?	Spearman correlations between fruit production and seasonal NAO indices per site.
Is fruit production best predicted by local weather variability or by seasonal NAO indices?	Generalized linear mixed models per species (with site as random factor) relating annual fruit production to seasonal temperatures, precipitations and NAO indices.
c) Synchrony in fruit production	
For a given year, is variability in fruit production among sites related to variability in weather conditions or to seasonal NAO indices?	Generalized linear mixed models per species (with site as random factor) relating annual CV of fruit production among sites to annual CV of seasonal temperatures and precipitations and to seasonal NAO indices.
Is fruit production (and weather) synchronized across sites within and among species?	Temporal synchrony of fruit production, temperature and precipitation among sites is calculated using Spearman correlations (i.e., correlation of time series A vs. time series B).
Is synchrony of fruit production and weather between sites spatially dependent?	Linear models relating synchrony of fruit production and seasonal temperatures and precipitations between sites to geographical distance.
Synchrony in fruit production between sites depends on synchrony in weather conditions and geographical distance?	Linear models relating synchrony of fruit production to synchrony of seasonal temperatures and precipitations and geographical distance between sites.

3. Results

3.1. Seasonal NAO effects on local weather

We found the seasonal NAO indices to be related to the weather of our sites (Figure 2). Positive winter NAO phases were related to warm and wet winters while positive spring NAO phases were related to warm and dry weather. Summer and autumn positive NAO's were related to cold and dry weather (Figure 2). On the other hand, we found no significant correlations among seasonal NAO indices either during the study period (2002 – 2010) or when analysing a larger period (1950 – 2014). In general, correlations among seasonal NAO phases were small and not significant, indicating little memory of the system.

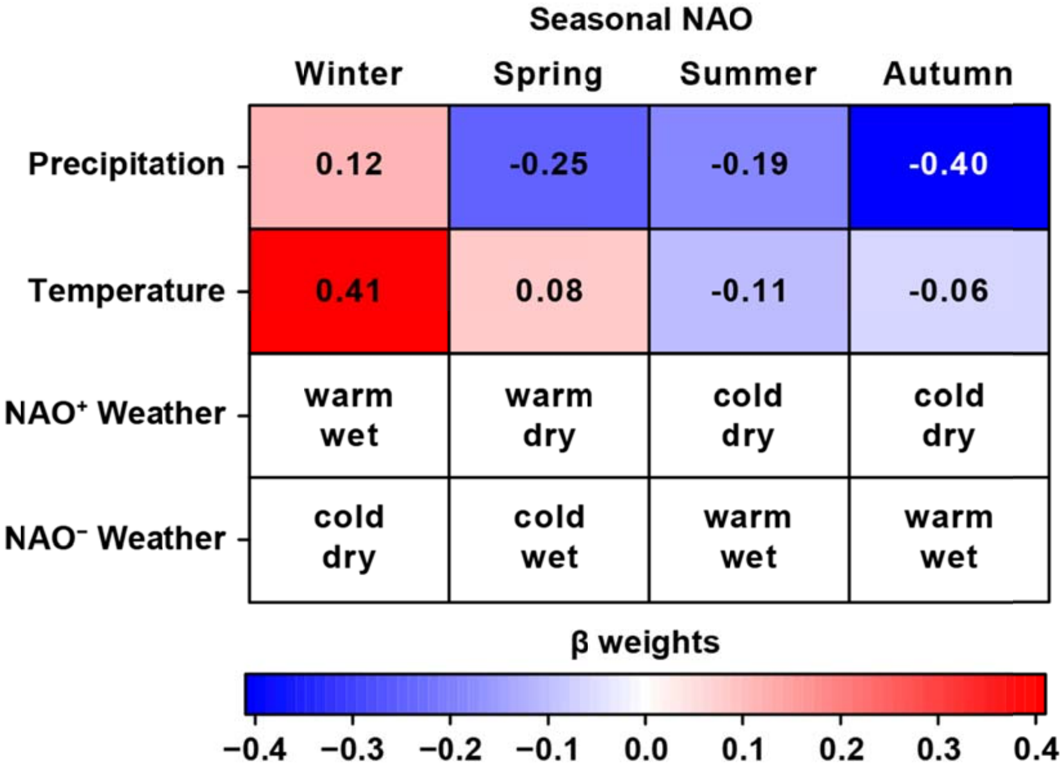

Figure 2: Relationship between seasonal NAO indices and weather during the period 2002 – 2010. Values are β weights calculated using generalised linear mixed models with random slopes. All coefficients were significant at the 0.001 level.

3.2. Interannual variability of fruit production

Both changes in weather and seasonal NAO indices were univariately correlated to fruit production for all tree species studied (Table 3). Fruit crop size in both *Quercus* species and *A. Alba* was negatively associated to the NAO phase of the autumn prior to fruit ripening whereas the three coniferous species presented strong positive correlations between fruit production and winter NAO (Table 3). Spring NAO was positively associated to fruit crop size in *A. alba*, *F. sylvatica* and *Q. robur* and summer NAO to *P. abies* and *F. sylvatica* (Table 3).

GLMM models predicting fruit crop size using seasonal local weather and seasonal NAO indices also showed a relevant role of the NAO phases in predicting interannual variability in fruit production (Table 4). In addition, seasonal NAO variables were usually the most important variables according to the ΔBIC. Following the univariate analysis, fruit crop size in *A. alba* was negatively related to winter NAO and positively related to spring NAO. The model also identified a significant negative relationship between winter precipitation and fruit production. According to the ΔBIC, both NAO variables were similarly important predicting variability in fruit production, and clearly more important than winter precipitation (Table 4). Large fruit crops in *P. abies* were associated to rainy winters, dry springs and dry (positive summer NAO) warm summers, being spring NAO and winter precipitation the most important variables. For *P. menziesii*, fruit crop size was positively related to cold and dry autumns (positive NAO_a), warm and wet winters (negative NAO_w) and warm spring temperatures, being the autumn NAO the most important variable in the model. Like for *P. abies*, fruit production in *F. sylvatica* was positively related to cold and dry autumns (positive autumn NAO and temperature) but also to dry and warm springs (spring NAO) and dry summers. Conversely *Q. petraea* and *robur* produced larger fruit crops after warm and wet autumns. Additionally, fruit production in *Q. robur* was positively correlated to wet summers and cold winters. Despite the best model predicting interannual variability for *Q. robur* did not show NAO to be a significant predictor, the second best model (differing only by 0.76 units of BIC from the best model in Table 4) included autumn NAO instead of winter temperature and let the ΔBIC of

Table 3: Spearman's correlations between seasonal (autumn, winter, spring and summer seasons prior to fruit production) NAO indices and fruit production (ρ ± standard error) per species and leaf types. *P* values tests whether average correlation coefficients differ from 0 (*t*-test). N indicates the number of forests per species. Only forests with 5 or more years of data were used in these analyses. The seasons are indicated using subscripts where "w" indicates winter, "sp" spring, "sm" summer and "a" indicates autumn.

	NAO_a		*P*		NAO_w		*P*		NAO_{sp}		*P*		NAO_{sm}		*P*		N
a) Species																	
Abies alba	-0.34 ± 0.10	ab	0.0031	**	-0.42 ± 0.11	b	0.0007	***	0.45 ± 0.09	ab	0.0003	***	-0.07 ± 0.07	b	0.5433		10
Picea abies	-0.22 ± 0.06	ab	0.0520	.	-0.42 ± 0.07	b	0.0007	***	0.13 ± 0.15	bc	0.2694		0.27 ± 0.12	ab	0.0141	*	10
Pseudotsuga menziesii	0.18 ± 0.25	a	0.2163		-0.48 ± 0.12	b	0.0026	**	-0.12 ± 0.20	c	0.4400		0.00 ± 0.22	ab	0.9772		6
Fagus sylvatica	0.06 ± 0.07	a	0.4089		0.03 ± 0.08	a	0.7266		0.61 ± 0.06	a	0.0000	***	0.36 ± 0.07	a	0.0000	***	21
Quercus petraea	-0.39 ± 0.08	b	0.0000	***	0.09 ± 0.10	a	0.2810		0.04 ± 0.10	bc	0.6014		-0.06 ± 0.06	b	0.4676		20
Quercus robur	-0.48 ± 0.11	b	0.0001	***	-0.14 ± 0.11	ab	0.2786		0.34 ± 0.11	abc	0.0092	**	-0.04 ± 0.15	b	0.7297		9
b) Leaf type																	
Conifers	-0.18 ± 0.08	a	0.0341	*	-0.43 ± 0.06	b	0.0000	***	0.20 ± 0.09	a	0.0253	*	0.08 ± 0.08	a	0.2900		26
Broadleaves	-0.21 ± 0.06	a	0.0005	***	0.02 ± 0.06	a	0.6490		0.33 ± 0.06	a	0.0000	***	0.12 ± 0.05	a	0.0250	*	50

the three predictors below 3 points. Therefore, seasonal NAO indices seemed to predict interannual variability of fruit production fairly well for all tree species studied.

Table 4: Summary of the models predicting fruit production per species. Standardised coefficients are shown as model estimates (β ± se). ΔBIC indicates the variable importance of the predictors and is calculated as the difference of BIC between the whole model and the model without the predictor of interest. Variance explained by the fixed factors (R^2_m) and by the whole model is also shown (R^2_c). All coefficients were significant at the 0.05 level. The seasons are indicated using subscripts where "w" indicates winter, "sp" spring, "sm" summer and "a" indicates autumn.

	β ± SE	**ΔBIC**	R^2_m	R^2_c
Abies alba				
$\mathbf{NAO_w}$	-0.40 ± 0.10	10.81		
$\mathbf{NAO_{sp}}$	0.42 ± 0.09	13.59		
$\mathbf{P_w}$	-0.29 ± 0.11	0.90		
Model			0.39	0.47
Picea abies				
$\mathbf{NAO_{sm}}$	0.31 ± 0.07	11.51		
$\mathbf{P_{sp}}$	-0.32 ± 0.12	2.62		
$\mathbf{P_w}$	0.49 ± 0.12	11.41		
$\mathbf{T_{sm}}$	0.38 ± 0.18	0.52		
Model			0.26	0.79
Pseudotsuga menziesii				
$\mathbf{NAO_a}$	0.39 ± 0.10	10.63		
$\mathbf{NAO_w}$	-0.29 ± 0.11	3.78		
$\mathbf{T_{sp}}$	0.57 ± 0.14	4.33		
Model			0.52	0.52

	β ± SE	**ΔBIC**	R^2_m	R^2_c
Fagus sylvatica				
$\mathbf{NAO_a}$	0.48 ± 0.07	34.37		
$\mathbf{NAO_{sp}}$	0.85 ± 0.07	88.22		
$\mathbf{P_{sm}}$	-0.22 ± 0.08	3.95		
$\mathbf{T_a}$	-0.16 ± 0.07	0.14		
Model			0.51	0.55
Quercus petraea				
$\mathbf{NAO_a}$	-0.20 ± 0.08	0.90		
$\mathbf{T_a}$	0.35 ± 0.08	11.89		
Model			0.21	0.21
Quercus robur				
$\mathbf{P_{sm}}$	0.30 ± 0.12	1.42		
$\mathbf{T_a}$	0.72 ± 0.16	14.34		
$\mathbf{T_w}$	-0.38 ± 0.15	2.45		
Model			0.26	0.28

3.3. Patterns of intra- and inter-specific synchrony in fruit production

Spring NAO was the most correlated variable explaining variability in annual fruit CV among species (Figure 3). Annual fruit CV was negatively related to spring NAO for all species except for *P. menziesii* while for *F. sylvatica*, *Q. robur* and *P. abies* the relationship was statistically significant (*P*=0.005, 0.012 and 0.041 respectively).

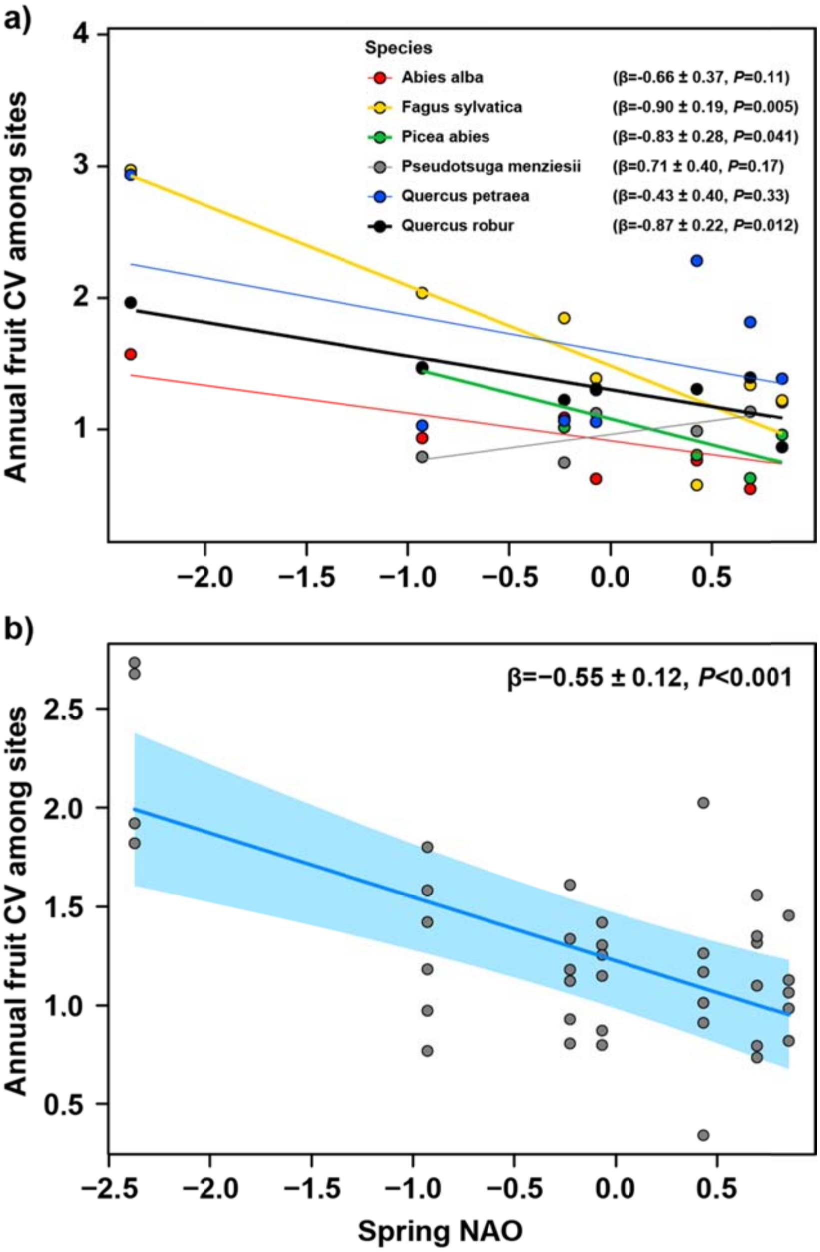

Figure 3: Relationship between annual fruit CV and spring NAO index for the six species under study. Thick lines indicate the relationship was significant at the 0.05 level. Lower layout shows the average relationship between fruit production CV and spring NAO index among species using generalized linear mixed models.

Table 5: a) Average synchrony (average Spearman's correlation of fruit production between sites: ρ ± standard error) in annual fruit production, seasonal temperature (T) and precipitation (P) between sites within species. b) Standardised slopes between synchrony and geographical distance (β ± se). Bold coefficients indicate values differ from zero at the 0.05 level (*t*-test). "N" indicates the number of pairwise comparisons. The seasons are indicated using subscripts where "w" indicates winter, "sp" spring, "sm" summer and "a" indicates autumn.

	A. alba	***P. abies***	***P. menziesii***	***F. sylvatica***	***Q. petraea***	***Q. robur***
a) Synchrony (ρ)						
Fruit NPP	**0.36 ± 0.07**	**0.31 ± 0.08**	0.19 ± 0.20	**0.60 ± 0.02**	**0.20 ± 0.04**	**0.29 ± 0.07**
T_w	**0.90 ± 0.01**	**0.89 ± 0.02**	**0.82 ± 0.02**	**0.89 ± 0.01**	**0.96 ± 0.01**	**0.94 ± 0.01**
T_{sp}	**0.73 ± 0.03**	**0.70 ± 0.05**	**0.72 ± 0.07**	**0.74 ± 0.02**	**0.85 ± 0.01**	**0.78 ± 0.03**
T_{sm}	**0.96 ± 0.01**	**0.95 ± 0.01**	**0.94 ± 0.02**	**0.94 ± 0.01**	**0.96 ± 0.01**	**0.95 ± 0.01**
T_a	**0.81 ± 0.02**	**0.82 ± 0.03**	**0.73 ± 0.06**	**0.69 ± 0.02**	**0.79 ± 0.01**	**0.85 ± 0.02**
P_w	**0.71 ± 0.04**	**0.80 ± 0.03**	**0.34 ± 0.15**	**0.44 ± 0.03**	**0.68 ± 0.02**	**0.38 ± 0.08**
P_{sp}	**0.62 ± 0.04**	**0.56 ± 0.07**	**0.62 ± 0.08**	**0.53 ± 0.03**	**0.58 ± 0.03**	**0.67 ± 0.03**
P_{sm}	**0.56 ± 0.04**	**0.61 ± 0.07**	**0.39 ± 0.14**	**0.42 ± 0.03**	**0.77 ± 0.01**	**0.65 ± 0.05**
P_a	**0.61 ± 0.05**	**0.74 ± 0.03**	**0.57 ± 0.13**	**0.49 ± 0.03**	**0.68 ± 0.02**	**0.59 ± 0.04**
b) β with distance						
Fruit NPP	-0.20 ± 0.16	-0.35 ± 0.21	-0.39 ± 0.38	-0.06 ± 0.08	-0.01 ± 0.09	**-0.71 ± 0.13**
T_w	**-0.65 ± 0.13**	**-0.69 ± 0.16**	-0.29 ± 0.39	**-0.54 ± 0.07**	**-0.33 ± 0.08**	**-0.79 ± 0.12**
T_{sp}	**-0.62 ± 0.13**	**-0.44 ± 0.20**	**-0.75 ± 0.27**	**-0.67 ± 0.06**	**-0.70 ± 0.06**	**-0.82 ± 0.11**
T_{sm}	**-0.52 ± 0.14**	-0.22 ± 0.22	-0.06 ± 0.41	**-0.41 ± 0.07**	-0.07 ± 0.09	**-0.65 ± 0.14**
T_{fall}	**-0.51 ± 0.14**	**-0.56 ± 0.19**	-0.02 ± 0.41	**-0.44 ± 0.07**	**-0.32 ± 0.08**	**-0.50 ± 0.16**
P_w	**-0.86 ± 0.08**	**-0.45 ± 0.20**	**-0.75 ± 0.27**	**-0.58 ± 0.06**	**-0.21 ± 0.08**	**-0.68 ± 0.14**
P_{sp}	**-0.75 ± 0.11**	-0.31 ± 0.21	-0.03 ± 0.41	**-0.35 ± 0.07**	**-0.37 ± 0.08**	-0.04 ± 0.19
P_{sm}	**-0.41 ± 0.15**	**-0.67 ± 0.17**	-0.54 ± 0.34	**-0.36 ± 0.07**	**-0.47 ± 0.08**	**-0.61 ± 0.15**
P_{fall}	**-0.79 ± 0.10**	**-0.64 ± 0.17**	-0.30 ± 0.39	**-0.58 ± 0.06**	**-0.60 ± 0.07**	**-0.79 ± 0.12**
N	38	22	8	163	135	30

Among forests, *F. sylvatica* was the most synchronized species producing fruits, reaching a mean synchrony among sites of 0.60 ± 0.02. On the contrary, *P. menziesii* did not present a statistically significant synchrony in fruit production among sites (Table 5, a). *A. alba*, *P. abies*, *Q. petraea* and *Q. robur* presented also an important synchrony in fruit production. However, synchrony in fruit production was significantly lower than synchrony in most of the seasonal weather variables (temperature and precipitation) for most of the species (Table 5, a). Although all species (except *P. menziesii*) presented a strong spatial correlation with weather synchrony (Table 5, b), the synchrony of fruit production was hardly spatially dependent using univariate regressions. Only *Q. robur* presented a statistically significant slope between synchrony in fruit production and distance between plots and for some species like *F. sylvatica* and *Q. petraea*, the slope coefficients were very close to zero (Table 5, b), being statistically different to most of the distance × weather correlation slopes (t-tests, $P<0.05$). *A. alba*, *P. abies*, *P. menziesii* and *Q. robur* presented slopes between fruit production and distance that did not significantly differd (t-tests, $P>0.05$) from most of the weather × distance relationships.

Among species, synchrony in fruit production decreased considerably and some combinations even presented significantly negative correlations, like *F. sylvatica* versus *P. menziesii and Q. petraea* (Table 6). Overall, synchrony of fruit production among was similar between leaf types (Table 6; *t*-test, $P>0.05$).

Our models relating synchrony in fruit production with synchrony in weather variables indicated that seasonal weather had a limited impact driving intraspecific synchrony among sites (Table 7). Synchrony of weather conditions between sites were only significant drivers of synchrony in fruit production for *A. alba* (autumn temperature), *F. sylvatica* (winter precipitation) and *Q. petraea* (summer precipitation) (Table 7). The only significant relationship for synchrony in fruit production of *Q. robur* was a negative relationship with distance between sites (Table 7) similar to the results obtained in univariate analyses (Table 5). *P. abies* did not present any significant relationship between synchrony in fruit production and synchrony in weather conditions.

Table 6: Average synchrony (Spearman's ρ ± standard error) between sites within and among species. Comparisons among different leaf types and among all sites are also shown. Bold coefficients indicate values differ from zero at the 0.05 level (*t*-test). The number of comparisons is shown below each coefficient between brackets. Only comparisons with 5 or more years of shared data were used.

	A. alba	***P. abies***	***P. menziesii***	***F. sylvatica***	***Q. petraea***	***Q. robur***
A. alba	**0.36 ± 0.07** **(38)**	0.07 ± 0.07 (53)	0.10 ± 0.06 (23)	**0.20 ± 0.03** **(161)**	**0.12 ± 0.03** **(116)**	**0.26 ± 0.03** **(81)**
P. abies	0.07 ± 0.07 (53)	**0.31 ± 0.08** **(22)**	0.02 ± 0.06 (24)	**0.08 ± 0.04** **(130)**	**0.09 ± 0.04** **(113)**	0.04 ± 0.04 (46)
P. menziesii	0.10 ± 0.06 (23)	0.02 ± 0.06 (24)	0.19 ± 0.20 (8)	**-0.10 ± 0.05** **(65)**	-0.07 ± 0.05 (86)	-0.13 ± 0.10 (19)
F. sylvatica	**0.20 ± 0.03** **(161)**	**0.08 ± 0.04** **(130)**	**-0.10 ± 0.05** **(65)**	**0.60 ± 0.02** **(163)**	**-0.12 ± 0.03** **(279**	**0.10 ± 0.04** **(141)**
Q. petraea	**0.12 ± 0.03** **(116)**	**0.09 ± 0.04** **(113)**	-0.07 ± 0.05 (86)	**-0.12 ± 0.03** **(279)**	**0.20 ± 0.04** **(135)**	**0.28 ± 0.04** **(97)**
Q. robur	**0.26 ± 0.03** **(81)**	0.04 ± 0.04 (46)	-0.13 ± 0.10 (19)	**0.10 ± 0.04** **(141)**	**0.28 ± 0.04** **(97)**	**0.29 ± 0.07** **(30)**

Comparisons among leaf types	
Broadleaves	**0.17 ± 0.02** **(845)**
Conifers	**0.17 ± 0.03** **(168)**
All species	**0.12 ± 0.01** **(1830)**

Table 7: Summary of the models relating synchrony (ρ) of fruit production between sites with synchrony in weather conditions and NAO products (NAO_{-p}). Coefficients are β weights ± standard error. The R^2 shows the total variance explained by the model. All coefficients were statistically significant at the 0.05 level. Only comparisons with 5 or more years of shared data were used in the models. Seasonal temperature and precipitation variables are indicated, respectively, as "T" and "P". The seasons are indicated using subscripts where "w" indicates winter, "sp" spring, "sm" summer and "a" indicates autumn.

	A. alba	***P. abies***	***F. sylvatica***	***Q. petraea***	***Q. robur***
Distance					**-0.71 ± 0.13**
T_a	**0.45 ± 0.15**				
P_w			**0.20 ± 0.08**		
P_{sm}				**0.20 ± 0.09**	
R^2	**0.20**	**-**	**0.04**	**0.04**	**0.50**
Comparisons	**38**	**22**	**163**	**135**	**30**

4. Discussion

4.1. Teleconnection indices as biological predictors

Our results fully support the hypothesis that teleconnection indices correlate better with biological processes than local weather (Ottersen *et al.*, 2001; Hallett *et al.*, 2004). All species presented statistically significant relationships with seasonal NAO indices (Figure 3, Table 3, Table 4) which highlight the importance of the NAO controlling ecological processes by affecting weather conditions (Figure 2) through large spatial scales. These results are thought to emerge because of the fact that organisms do not respond to single environmental variables, but rather to a combination of them. In this sense, the NAO indices act as *weather packages* (i.e., temperature, precipitation, humidity, wind, radiation, pressure) reducing spatio-temporal variability in weather conditions into one single index (Stenseth *et al.*, 2003) influencing weather over continental scales. The characteristics of the NAO (or other teleconnection) indices (weather packages influencing very large geographical scales) make them very suitable candidates as variables for testing the Moran effect.

4.2. Controls of interannual variability of fruit production

Our results highlighted a contrasting effect of the seasonal NAO indices on fruit production for coniferous and broadleaved species: while coniferous species were mainly negatively correlated to winter NAO (i.e., associated to dry and cool winters), fruit production of broadleaved species presented positive relationships with spring NAO (it was associated to warm and dry springs) and negative relationships with autumn NAO (it was associated warm and wet autumns) (Table 3, Table 4) although these relationships can be altered by local weather (i.e., positive relationship between winter precipitation and fruit production for *P. abies* in Table 4). Those differences are, in part, due to the differences in leaf habit affecting the timing on which weather influences fruit production, since our studied coniferous species are all evergreen (main effect during winter) and the broadleaved are all deciduous (main effect during spring).

In evergreen species, cold winters may delay the growing season and thus the onset of flowering (Frenguelli & Bricchi, 1998; García-Mozo *et al.*, 2002; Stöckli & Vidale, 2004). Because of cold weather conditions, delayed pollination period has been previously reported to positively affect fruit production in the evergreen *Quercus ilex* (Fernández-Martínez *et al.*, 2012). Additionally, cold winter weather may also provide the necessary chilling requirements for conifers to reach a complete dormancy period (Clancy *et al.*, 1995), which is needed to survive unfavourable environmental conditions. On the contrary, higher winter temperatures might entail higher metabolic costs for trees, reducing the potential reserves to invest in reproduction. Therefore, this reasoning would be in line with the *resource matching* hypothesis since trees would produce fruits as a response to the available resources (Table 1).

On the other hand, dry and warm spring weather (when pollination occurs) has been demonstrated to facilitate pollen dispersal, since temperature increases pollen release to the atmosphere and precipitation removes it (García-mozo *et al.*, 2006; Fernández-Martínez *et al.*, 2012; Kasprzyk *et al.*, 2014). We, thus, suggest that the positive association between fruit production in deciduous broadleaved species (and *A. alba,* see Table 3, Table 4) and the spring NAO phase emerges because dry and warm weather facilitates pollen release and therefore fertilization of female flowers, supporting the *pollination efficiency* hypothesis (Table 1).

Fruit crop size for *Q. petraea*, (and also *Q. robur* and *A. alba*, see Table 3) was correlated to warm and wet autums. During warm and wet autumns ($-NAO_a$) leaf senescence and the start of the dormancy period is delayed because of the strong control that temperature exerts on them (Vitasse *et al.*, 2009; Estiarte & Peñuelas, 2015). Delayed leaf senescence increases the growing period which allows trees to accumulate resources just after most of the fruits are already mature. This additional acquisition of resources at the end of the growing season could be spent during next spring entailing a larger flowering effort, thus also agreeing with the *resource matching* hypothesis (Table 1).

Dry and cool summer weather ($+NAO_{sm}$) increased fruit crop size in *P. abies* and *F. sylvatica* (Table 3). Because species from colder and wetter environments are more sensitive to changes in temperature than in water availability (Fernández-Martínez *et al.*, 2014), high summer temperatures may lead to photoinhibition and stomatal closure and, therefore, reduce the photosynthetic capacity of trees, constraining the resources available to allocate to ripening fruits. This reasoning concerning the effects of the summer NAO on fruit production further supports the *resource matching* hypothesis for *P. abies and F. sylvatica* species (Table 1).

Overall, our results point out to proximal causes (weather related to increased productivity or better pollination conditions) as some of the most plausible mechanisms to explain interannual variability in fruit production. They also highlight a possible role of the *resource matching* combined to the *pollination efficiency* hypotheses (Table 1), especially for the broadleaved species studied here, as potential drivers of interannual variability in fruit production of European forests. It is very likely that mechanisms proposed by these two hypotheses are required to a certain degree in order to explain large interannual variability in fruit crops and its synchrony. The importance of each of them will surely depend on the species under study and the site characteristics (Fernández-Martínez *et al.*, 2012).

4.3. Controls of synchrony of fruit production

Whether synchrony in fruit production happens because of the Moran effect or the pollen coupling is hard to tell in most cases because both hypotheses generate similar patterns of spatial synchrony and are not mutually exclusive (Liebhold *et al.*, 2004; Koenig & Knops, 2013). However, some predictions, emerging from both hypotheses, can help to distinguish them. First, synchrony in fruit production because of *pollen coupling* should not happen farther than a few hundred kilometres at most, while the Moran effect can easily reach hundreds and even thousands of kilometres (Koenig & Knops, 2013). According to this prediction, our results would suggest that the Moran effect is responsible for the synchrony in fruit production among forests, some of them separated by hundreds of kilometres from each other. However, this assumption does not allow us to dismiss the *pollen coupling* hypothesis since our database consists of forests and not individual trees, and hence, *pollen coupling* might still be acting at the local scale.

On the other hand, the anti-synchrony in fruit production between *F. sylvatica* and *Q. petraea* (Table 6), two potentially co-existing species, may suggest some kind of strategy for avoiding large fruit crops in the same years. This would have no sense for avoiding seed predation (see the *predation satiation* hypothesis (Silvertown, 1980; Espelta *et al.*, 2008)) but it could benefit pollination competition because mainly pollen from the own species would reach female flowers. If this hypothetical mechanism were to happen, it would support that *pollen coupling* synchronizes fruit production. Nonetheless, synchrony between non-hybridizing species cannot take place because of *pollen coupling* and we did find significant synchrony among species that do not hybridize (Table 6). This result would further support the Moran effect, especially when combined with results from models predicting interannual variability in fruit production suggesting the seasonal NAO indices as common drivers of interannual variability.

The Moran effect also predicts similar relationships of synchrony in fruit production and weather variables with the distance between sites (Koenig & Knops, 2013). Our results agree with this prediction for *A. alba*, *P. abies*, *P. menziesii* and *Q. robur,* thus further supporting the Moran effect. However, our findings for *F. sylvatica* and *Q. petraea*, disagree with this prediction because for these species synchrony in fruit production presented no relationship with distance at all (Table 5). Since *F. sylvatica* presented also interannual variability in fruit production to be linked to spring weather favouring pollen dispersal, it is likely that *pollen coupling* also plays a role synchronizing fruit production in these forests and possibly also in *Q. petraea* forests (Table 1). On the other hand, synchrony in weather conditions was correlated to synchrony in fruit production for *A. alba*, *F. sylvatica* and *Q. petraea* suggesting a potential role of weather synchronizing fruit crop sizes among sites. However, because none of the variables involved predicting synchrony in fruit production predicted also interannual variability in fruit production and because of the little variance explained by the synchrony models, we should be very careful with the interpretation of these results. Nonetheless, given the fact that the NAO is in part responsible for the interannual variability in weather conditions, these results would indicate that the NAO acts as a synchronizing agent among sites and that the Moran effect is probably the main factor synchronizing fruit production of forests from central Europe (Table 1).

Hence, both hypotheses, the Moran effect and the *pollen coupling*, seem to play a role synchronizing fruit production, but at different levels. Whereas the Moran effect presents a clear signal at continental scales, the *pollen coupling* seems to be restricted to the local or the nearby environment. Moreover, according to our results, the signal of the Moran effect seems to be more common than the signal for the *pollen coupling*, which we found to be restricted mainly to the deciduous broadleaved species studied.

Overall, our results highlight that weather packages, such as the NAO index, can help to better predict ecological processes at wide geographical scales, which is particularly useful in order to test the Moran effect. We also found that the synchronising effect of weather is more likely to be driven by proximal causes driving interannual variability than by the effect of weather as a cue since the relationships found between weather and fruit production are likely to affect tree resources or to enable favourable weather conditions to pollination. In addition, given the considerable variance explained by the models explaining interannual variability in fruit production, our results point out that weather variability should be one of the most important factors driving interannual variability and synchrony in fruit production (Fernández-Martínez *et al.*, 2015).

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Chapter 7

Masting in oaks: disentangling the effect of flowering phenology, airborne pollen load and drought

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Abstract

Quercus species exhibit an extreme interannual variability in seed production often synchronized over large geographical areas (masting). Since this reproductive behaviour is mostly observed in anemophilous plants, *pollination efficiency* is suggested as one hypothesis to explain it, although *resource-based* hypotheses are also suggested as alternatives. We analysed the effect of flowering phenology, airborne pollen presence and meteorological conditions in the pattern of acorn production in mixed evergreen-deciduous oak forests (*Quercus ilex* and *Q. pubescens*) in NE Spain for twelve years (1998-2009). In both oaks, higher temperatures advanced the onset of flowering and increased the amount of airborne pollen. Nevertheless, interannual differences in pollen production did not influence acorn crop size. Acorn production was enhanced by a delay in flowering onset in *Q. ilex* but not in *Q. pubescens*. This suggests that in perennial oaks a larger number of photosynthates produced before flowering could benefit reproduction while the lack of effects on deciduous oaks could be because these species flush new leaves and flowers at the same time. Notwithstanding this effect, spring water deficit was the most relevant factor in explaining interannual variability in acorn production in both species. Considering that future climate scenarios predict progressive warmer and dryer spring seasons in the Mediterranean Basin, this might result in earlier onsets of flowering and higher water deficits that would constrain acorn production.

1. Introduction

Mast seeding (or *masting*) is one of the most peculiar reproductive behaviours observed in perennial plants with high interannual variability in seed crops and high levels of synchronization in seed production at the population- and community-level, over large geographical areas (Kelly and Sork, 2002; Shibata et al., 2002; Espelta et al., 2008). This process has important consequences not only for the recruitment of the species that exhibit this reproductive behaviour (e.g. seedling establishment may be limited to mast years, see Negi et al., 1996) but also for a myriad of organisms in other trophic levels, whether directly or indirectly related to seed availability: e.g. direct consumers of seeds (insects, birds, small and large mammals), predators of seed consumers and parasites (see examples in McShea, 2000; Cahill and Llimona, 2004; Espelta et al., 2008; Kelly et al., 2008).

Since masting is mostly observed in wind-pollinated (anemophilous) rather than insect-pollinated (entomophilous) plants (Smith et al., 1990), *pollination efficiency* has been suggested to be one of the potential factors triggering such a highly variable seed production (Norton and Kelly 1988; Smith et al., 1990). This hypothesis, based on *economy of scale*, states that, in order to ensure pollination, in wind-pollinated species it is more efficient to invest a large amount of resources so as to saturate the atmosphere with pollen in a given year than to produce similar but lower amounts of pollen year after year. In this scenario, *pollen coupling* would be the factor synchronizing reproduction among individuals within the population (Isagi et al., 1997, Satake and Iwasa, 2000): the development of female flowers after a massive pollination event would result in a depletion of resources for all individuals in the population, leading to the synchronization of their reproduction pattern. Another hypothesis, based on *economies of scale*, is the *predator satiation* hypothesis (Silvertown, 1980) considered to be one of the most prominent explanations supporting masting as a selective response (Kelly and Sork, 2002).

Alternatives to the adaptive (*economies of scale*) hypotheses are the *resource matching* and the *resource accumulation* hypotheses (Norton and Kelly, 1988; Sork et al., 1993; Isagi et al., 1997; Kelly and Sork 2002; Camarero et al., 2010). The former states that seed production depends mostly on the amount of resources available, meaning that better environmental conditions for photosynthesis during the growth season will later provide a larger amount of seeds. The resource accumulation suggests that trees accumulate resources over several years until a threshold is reached and a massive reproductive event occurs. In this case, a large number of seeds will be produced with a certain periodicity despite the weather effect. In these two hypotheses based on resource availability, the *Moran Effect* (Ranta et al., 1997) would explain intra- and inter-specific synchronization among individuals: i.e. plants would synchronize their reproductive efforts in response to certain *environmental cues*, such as particular meteorological events (e.g. temperatures, droughts or even fire episodes; Kelly and Sork, 2002).

Several previous studies have aimed at assessing whether *pollination efficiency* or *resource availability* is the main factor driving masting. However, very few of them have been able to simultaneously use both aerobiological (pollen) and meteorological datasets for the same site (although see García-Mozo et al., 2007). Indeed, most studies have focused either on *pollination efficiency* (see Knapp et al., 2001; Kon et al., 2005) or on the *resource-based* hypotheses (see Sork et al., 1993; Espelta et al., 2008; Camarero et al., 2010; Pérez-Ramos et al., 2010). Moreover, in addition to the lack of long-term studies including both pollen and meteorological data, the potential effect of the phenology of flowering on variable seed production has seldom been explored. Despite this, it has already been observed that differences in flowering onset across years may influence the final number of seeds produced due to differences in the availability of outcross pollen, extreme climate events or seed predator activity (Ollerton and Díaz, 1999; Lacey et al., 2003).

The main aim of this study was to test whether pollen availability (*pollination efficiency*) or resources (*resource matching or accumulation* hypotheses) are the major forces driving masting in two Mediterranean oaks (*Quercus ilex* and *Quercus pubescens*), and to evaluate whether interannual differences in the flowering phenology may also influence this process. Furthermore, as Kelly and Sork (2002) suggested that plants in poorer habitats should present more pronounced masting behaviour because they would need longer periods to recover from a massive reproduction event, we have also analysed the temporal pattern in acorn crop size among stands differing in soil water availability. To address these questions we have used a dataset from a monitoring survey in the natural Park of Collserola (NE Spain), which includes: 17 years of airborne pollen load (1994-2010), 12 years of acorn crop production (1998-2009) and three years (2007-2009) of intense monitoring of flowering phenology in order to check for the accuracy of the pollen data as a proxy of flowering phenology. Understanding whether pollen availability or environmental factors govern seed production is important not only for explaining masting itself but also for gaining insight into the potential effects of climate change scenarios on tree reproduction. In the specific case of Mediterranean oak forests, it has been predicted that the increase in drought episodes in the Mediterranean region (IPCC, 2007) will produce changes in plant phenology (Peñuelas et al., 2002; García-Mozo et al., 2006), increase airborne pollen availability (Knapp et al, 2001; García-Mozo et al., 2006) and also cause a higher rate of seed abortion (Espelta et al., 2008; Sanchez-Humanes and Espelta, 2011).

2. Materials and methods

2.1. Study area and species

This study was carried out in the Collserola Massif (Lat: 41° 26' N, Long: 2° 06' E), located in the northeast of the Iberian Peninsula. The massif is situated parallel to the sea coast (southwest – northeast) leading to an orientation that entails substantial differences between slopes: i.e. southeastern (dryer, and with higher irradiation) and northwestern (wetter, more continental and shady). While the southeastern slopes are mainly characterized by mixed pine and holm-oak forests (*Pinus halepensis and Quercus ilex*), the northwestern slopes shows a lower density of pines and a higher presence of *Quercus ilex* and winter-deciduous oaks such as *Quercus pubescens* (Bolós and Vigo, 1990). Interestingly, in spite of their different leaf habit, these two oaks show a high interannual variability in terms of acorn crops (Espelta et al., 2008), withstand the same seed predators (Espelta et al., 2009 a, b), and share a similar regeneration niche (Espelta et al., 1995).

The climate is typically Mediterranean with a strong sea influence: mild winters, strong water stress in summer, and wet, torrential springs and, especially, autumns (Martín Vide, 1992). For the current standard climatological normal period (1991 – 2010), the Fabra Observatory, located in Collserola at 415 masl (metres above sea level), presents a mean annual temperature of 15.7 ± 1.4°C, with January being the coldest month (8.5 ± 0.6°C) and August the hottest one (24.3 ± 0.7 °C).

Mean annual precipitation is 613.8 ± 34.0 mm with a CV of 0.25, considered to be a high interannual variability (Martín Vide, 2002). Interestingly, Collserola has experienced an important increase in the mean temperature since 1914 (1.6 ± 0.6ºC) which has been drastically accelerated in recent years (Figure S7.1). Although annual rainfall shows no trend, probably due to an extremely high interannual variability pattern, the annual water deficit has increased by about 358.0 ± 229.4 mm since 1914 owing to the rise in temperatures.

2.2. Experimental design and sampling

2.2.1. Airborne pollen data

We used data from the Catalan Aerobiological Network (XAC) using Hirst volumetric spore-traps from two sampling stations close to our study area (Figure S7.2) and representative of the southeastern and northwestern slopes, respectively: Barcelona (Lat: 41º 23' N, Long: 2º 9' E at 31 masl) and Bellaterra (Lat: 41º 30' N, Long: 2º 6' E at 130 masl). In this study, we used airborne pollen concentration as a proxy of forest pollen production, as demonstrated in previous studies (see Estrella et al., 2006 for *Betula*, *Poaceae* and *Artemisia sp.* and Jato et al., 2007 for *Quercus sp.*). The morphological differences in the pollen grains captured in the Hirst traps make it possible to distinguish between evergreen and deciduous oaks (Belmonte and Roure, 1991; Jato et al., 2007), which, in our study area, mostly correspond to the evergreen *Q. ilex* and the deciduous *Q. pubescens*.

From the daily airborne pollen concentration dataset, several variables were calculated: i) the start of the pollination period (henceforth DI), defined as the first day that 1 pollen grain m^{-3} was captured and the following days recorded one or more pollen grains m^{-3} (García-Mozo et al., 2002, 2006): ii) the peak date, defined as the day with the maximum airborne pollen concentration: iii) the end of the pollen season, defined as the date when no pollen grains were recorded for more than one day and, iv) pollen production until the peak (henceforth pollen production), calculated as the sum of all pollen records previous to the pollen peak date. This variable is often used instead of the total pollen production in a season to avoid inflating the amount of pollen produced by counting remobilized pollen once most female flowers have been pollinated or wilted. Pollen production until the peak has been found to be better correlated with seed production (García-Mozo et al., 2007).

2.2.2. Phenological data

To check whether pollen records accurately indicated the phenological pattern of flowering of oaks in Collserola, we compared the temporal pattern of airborne pollen data from Barcelona and Bellaterra stations with a very thorough survey of flowering phenology carried out in a field station (Can Balasc) in Collserola in 2007, 2008 and 2009 (see Sánchez-Humanes, 2009). The visual survey of the phenology of male flower (catkin) production was carried out every 15 days from February to September in 2007, 2008 and 2009 in 20 *Q. pubescens* and 20 *Q. ilex* trees randomly selected (see Sánchez-Humanes, 2009 for further details). For the purposes of this study the variables calculated were the mean values among trees for: i) the date of the onset of flowering; ii) the percentage of the tree canopy bearing catkins (visually estimated); iii) the flowering peak, considered to be the date when the maximum proportion of the canopy bearing catkins was recorded; and iv) the end of the pollination period, regarded as the date when trees no longer presented any open flowers in their canopies.

2.2.3. Acorn crop data

Acorn crop records came from a monitoring program carried out from 1998 to 2009 in 17 sampling sites (Figure S7.2) in mixed *Q. ilex* and *Q. pubescens* forest stands at least 50 years old according to aerial photographs taken in 1956 (CECAF 1956). Plots were distributed in different topographic conditions along the Collserola Massif (from 141 to 415 masl) and with a mean distance of 4.7 ± 2.4 km between them (Espelta et al. 2008). In these plots, *Q. ilex* had a higher density than *Q. pubescens* (1357 ± 219 stems ha^{-1} vs. 294 ± 71 stems ha^{-1} respectively; paired t-test: $t = 4.89$; p-value $=0.00024$). At the beginning of the study (1998), 510 trees were tagged (15 individuals from each oak species per plot) and the number of branches per tree was estimated using a regression model between crown projection and number of branches previously constructed for a sub-sample of trees. From 1998 to 2009 we counted acorn production on four branches per tree at the peak of the acorn crop (i.e. September). Then we estimated the total number of acorns produced per tree by multiplying the mean acorn production per branch and the number of branches per tree (see Espelta et al. 2008 and Espelta et al. 2009a for further details on this sampling protocol). From these data we calculated, on a per year basis, the percentage of reproductive trees (out of those producing acorns at least once during the study period), mean mature acorn crop size (number of acorns) per tree (excluding those individuals which never produced acorns during the study period), and annual interindividual variability in acorn crop size, calculated as the coefficient of variation of the annual acorn crop among individuals. In addition, we calculated the population variability per plot on acorn production over the 12 years studied as an estimation of the inter-year variability (CV). The existence of a potential spatial autocorrelation in mean mature acorn production per tree among plots was checked by means of Mantel tests in both oaks. None of them presented spatial autocorrelation (*Q. ilex*: r = 0.13; p-value = 0.1565, *Q. pubescens*: r =0.10; p-value = 0.2019).

2.2.4. Meteorological and climate data

Two weather stations were used in this study in order to represent both slopes of the Collserola Massif: the Fabra Observatory, located at 415 masl on the southeastern slope of Collserola (Lat: 41° 25' N, Long: 2° 7' E) and near Barcelona's aerobiological station (4.4 km), and the Cerdanyola del Vallès weather station, at 84 masl on the northwestern slope of Collserola (Lat: 41° 29' N, Long: 2° 7' E) and near Bellaterra's aerobiological station (2.4 km). Data from the Fabra Observatory include daily rainfall, maximum and minimum temperature data from 1914 to 2010. The Cerdanyola del Vallès weather station compiled the daily rainfall, mean, maximum and minimum temperatures from 1998 to 2010. The missing values in this series for the period included in the present study (1994 to 1998) were filled in with data from the Fabra Observatory, previously homogenized by the Cerdanyola weather station. This homogenization was carried out by applying a monthly additive term for temperatures and a multiplicative factor for rainfall extracted from the difference between both stations on climate variables for the period 1999 to 2010 (see Martín Vide, 2002).

In order to obtain an indicator of soil water availability, in each of the 17 sites where acorn production was monitored, we calculated the climatic water deficit (potential evapotranspiration – real evapotranspiration in mm; Thornthwaite, 1948). This index was calculated for every site using mean monthly rainfall and temperature climate data taken from the Digital Climate Atlas of Catalonia (Pons, 1996; Ninyerola et al., 2000).

To test the effect of previous meteorological conditions on the onset of flowering and pollen production, we calculated the sum of rainfall and mean maximum, minimum and average temperatures for January, February and March, as well as for the entire period. We analysed the effect month by month and throughout the whole pre-flowering period so as to clarify whether there was a stronger influence by the temperatures of any particular month or by all pre-flowering conditions. Likewise, we analysed the effect of meteorological conditions (temperature and rainfall) during the pollination (flowering) season on the amount of pollen produced. We used multiple stepwise (forward) regressions analyses with the aerobiological parameters being the dependent variables and the meteorological parameters the independent ones.

Different factors are known to affect the onset of flowering onset such as photoperiod, rainfall and evapotranspiration (García-Mozo et al., 2002) although temperature seems to be the most influential one (García-Mozo et al. 2006). To evaluate this influence, we used Growing Degree Days (GDD) as an estimation method of heat accumulation. GDD were calculated for multiple threshold temperatures (from 0 to 11°C) using the methodology proposed by Snyder (1985). The most accurate threshold temperature was established by taking into account the best linear model forced through the origin in a regression analysis (observed vs. predicted flowering dates) and the lowest Root Mean Square Error (RMSE) of the predicted values minus the observed days for all of the years studied (see García-Mozo et al., 2002).

2.3. Data analysis

In order to test the extent to which the airborne pollen load reflects the phenological pattern of flowering, Spearman correlations were used to evaluate the correlation between the percentage of flowering canopy (covered by catkins) and the amount of pollen collected.

To test the relation between aerobiological parameters and mature acorn production, plots were classified into two groups: the plots nearest Barcelona (located in Collserola's southeastern slope) and those nearest Bellaterra (located in the northwestern slope). We selected this classification owing to the known negative effect of increasing distance from the source over the airborne pollen concentrations (Knapp et al., 2001; Estrella et al., 2006). We assume that the phenological and pollen signal of a plot would be stronger at the nearest aerobiological station. These classification criteria resulted in 5 plots being classified as nearest Barcelona and 12 nearest Bellaterra (Figure S7.2).

To test whether aerobiological variables or resource availability (water deficit) were the main factors affecting the acorn crop, we used multiple stepwise (forward) regressions for both *Quercus* species and the two slopes (Barcelona, Bellaterra), with mean acorn crop per tree, percentage of productive trees and interindividual acorn crop variability being the dependent variables and date of the onset of flowering, pollen production and annual spring water deficit the independent ones. We used annual spring water deficit from April to June in this analysis, calculated following Thornthwaite's method (1948), after we ran a preliminary analysis comparing water deficit for all months of every year and acorn production by means of correlation analyses. As water deficit in April, May and June were the most correlated months, we used the accumulated spring water deficit during these three months for comparing the effects of aerobiological variables on acorn production. In the multiple regression analyses, variables were $\log_{10}$-transformed if necessary to meet the assumption of normality. Additionally, the potential interaction between soil water availability and annual water deficit on acorn production was analysed by means of Generalized Linear Models. In this analysis, acorn production was entered for every year and every plot (N = 204) as the dependent variable assuming a Poisson distribution and soil water availability and annual spring water deficit as two continuous independent variables (ranging from 701.3 to 1111.8 and to 193.9 to 627.4 mm respectively). Nevertheless, to better show the results of this analysis we converted these two continuous variables into categories with three levels (low, middle and high according to the percentiles 1/3 and 2/3). We also tested the effect of soil water availability on the interannual variability in acorn production at the population level (CVp) by means of Pearson correlations.

Finally, to test the impact of previous acorn production on current crops we tested for temporal autocorrelation in acorn crops (see Sork et al., 1993) by means of an autocorrelation analysis (ACF) and a partial autocorrelation analysis (PACF). We decided to incorporate a PACF analysis to evaluate the impact of the *n*-lag acorn crops excluding the effect of the lower order lags in acorn crops. Thus, we used mean acorn crop per plot data rather than single-tree production to avoid pseudoreplication. We then calculated the autocorrelation coefficients of all plots until the sixth lag and performed a *t* – test (N=17) whose null hypothesis was no temporal autocorrelation (mean autocorrelation of the plots at a determined lag equals 0). Autocorrelation analyses were also done for spring water deficit from the Fabra Observatory and the Cerdanyola weather station for the same period to compare them with acorn crop autocorrelations. These aimed to determine whether the acorn crops' autocorrelations depend simply on weather or follow a different pattern which might suggest endogenous cycles of masting behaviour (Sork et al., 1993; Pérez-Ramos et al., 2010).

3. Results

For the two oak species (*Q. ilex*, *Q. pubescens*) and considering the airborne pollen data from the two slopes of the Collserola Massif (Barcelona, Bellaterra), there was a positive and significant relationship between the temporal trend of flowering intensity (i.e. percentage of the tree canopy bearing catkins) and the amount of pollen present in the atmosphere (Figure 7.1, Spearman Rank's correlations R > 0.60, p < 0.045 except for *Q. ilex* in Barcelona in 2008, R=0.57, p =0.084). Yet it must be noticed that there was a 15-day time lag between the two variables (i.e., the number of catkins in the canopy was related to the amount of pollen collected in the following 15 days). Concerning the phenology of flowering, the records of pollen presence in the atmosphere could be used as a proxy of the onset of flowering and a reliable indicator of the peak of flowering (Table 7.1). Conversely, the end of the flowering season could not be inferred from aerobiological data as the presence of pollen in the atmosphere extended far beyond this point, probably due to the continuous remobilization of already released pollen. According to the aerobiological data collected from 1994 to 2010 (Table 7.1), *Q. pubescens* shows an earlier start (c. 10 days) of the pollen season than *Q. ilex* (Paired t-tests; Barcelona: t = -6.13; p-value = 0.00001; Bellaterra: t = -3.53; p-value = 0.00277). Likewise, the peak pollen date and the end of the season occur earlier (respectively, c. 16 days and 47 days) in the former species (Paired t-test, Peak date, Barcelona: t = -3.20; p-value = 0.00554; Bellaterra: t = -8.55; p-value < 0.00001; End day, Barcelona: t = -7.00; p-value < 0.00001; Bellaterra: t = -7.74; p-value < 0.00001).

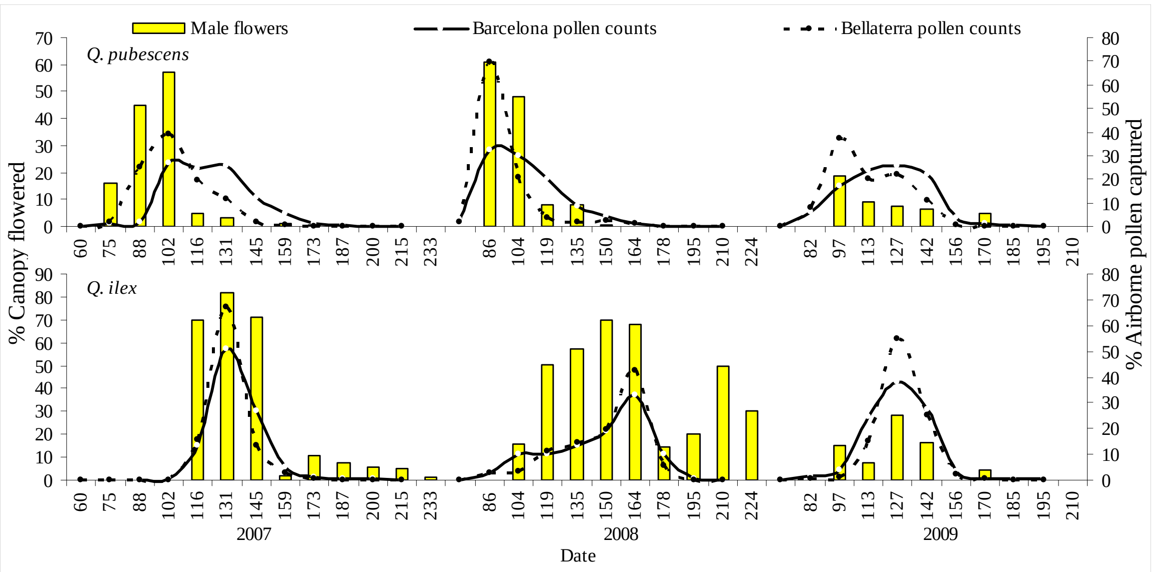

Figure 1: Evolution of the flowering period for years 2007 - 2009. Y-axis shows the percentage of the mean canopy covered with male flowers and the percentage of airborne pollen (in both aerobiological stations) collected during the 15 following days over the whole season. Dates are expressed in Julian days. BCN: Barcelona and BTT: Bellaterra.

For the two oaks, high pre-flowering temperatures from January to March resulted in an earlier beginning of the pollen season, with maximum temperatures being the main factor in Bellaterra and mean temperatures in Barcelona ($r < -0.62$; p-value < 0.01). In the more detailed analysis conducted on a monthly basis, March temperatures were the most relevant factor for starting the pollination season in both oaks ($r < -0.46$; p-value < 0.05). This relationship between temperature and the start of the pollen season was confirmed by the good fit of the *Growing Degree Day* models (Table S7.1).

Table 1: a) Julian dates for phenological observations and aerobiological data (2007 – 2009) for both species and aerobiological stations and b) Julian dates for aerobiological data (1994 – 2010). All values are mean ± standard error. BCN: Barcelona and BTT: Bellaterra.

a) 2007 – 2009		**Start**		**Peak**		**Ending**	
		Q. pubescens	***Q. ilex***	***Q. pubescens***	***Q. ilex***	***Q. pubescens***	***Q. ilex***
Flowering phenology		89 ± 4	116 ± 2	102 ± 4	144 ± 8	134 ± 7	162 ± 5
Pollen Counts	**BCN**	83 ± 6	97 ± 3	116 ± 13	145 ± 9	181 ± 8	207 ± 6
	BTT	82 ± 1	98 ± 8	106 ± 3	151 ± 9	170 ± 2	202 ± 7
b) 1994 – 2010							
Pollen Counts	**BCN**	84 ± 2	96 ± 3	125 ± 4	141 ± 2	170 ± 3	217 ± 5
	BTT	84 ± 2	94 ± 4	104 ± 3	140 ± 3	163 ± 2	206 ± 5

For the two species and sites, the best linear fit and the minimum Root Mean Square Error was obtained at a threshold temperature of 0ºC. Once the flowering season started, temperature continued to be the most relevant meteorological factor in determining the amount of pollen produced: i.e. in both oaks and localities (except for *Q. ilex* at the Barcelona aerobiological station): higher minimum temperatures during the pollination season resulted in larger amounts of pollen being released into the atmosphere. In contrast to the importance of temperature, rainfall barely influenced any of the characteristics of the pollination season (except for rainfall during March in Bellaterra, which delayed the onset of flowering of *Q. pubescens*, $r = 0.44$; p-value < 0.05).

The annual values of spring water deficit, airborne pollen load, and mature acorn crop size of *Q. ilex* and *Q. pubescens* are shown in Figure 7.2. In both oaks, the stepwise regression analyses revealed a negative relationship between the annual value of spring water deficit and the number of reproductive trees, acorn crop size per tree and a positive correlation with inter-tree variability in acorn production (Table 7.2). Interestingly, for *Q. ilex* (Table 7.2, *b*), we also found a positive effect of the date of the onset of flowering on the percentage of reproductive trees and the mature acorn crop. Conversely, pollen production barely influenced either the number of reproductive trees or mean acorn crop size per tree. Thus, spring water deficit seems to be the most relevant factor driving inter-annual variability in acorn crop size, as it was the only factor that entered in all models for both oaks (except for the acorn crop size in *Q. pubescens* in Barcelona, see Table 7.2*a*). However, when both spring water deficit and the date of the onset of flowering entered into a model, they presented similar contributions according to the comparison of the standardized partial β coefficients (t-tests, p-values > 0.05, see Table 7.2). Concerning the potential interaction between soil water availability (local water stress) and spring water deficit on acorn production, the analysis revealed a negative effect of both factors (soil water availability: Wald > 8.05; p-value < 0.01; spring water deficit: Wald > 61.97; p-value < 0.00001, Figure 7.3), but no interaction among them. In addition, population variability on acorn crop size per plot during the 12 years of monitoring did not present a significant relationship with the soil water availability.

Finally, for both oaks, the autocorrelation analysis (ACF, Table 7.3) showed a negative impact on the current acorn crop size of the first and the third lag and a positive effect of the sixth. However, when using partial autocorrelation coefficients (PACF, Table 7.3) both oaks showed a negative effect of lags 1 to 5 on the current acorn crop and only *Q. ilex* continued to be positively affected by the sixth lag's acorn production. Interestingly, water deficit exhibited a higher mean negative autocorrelation in the first lag than acorn production (Table 7.3) albeit differences not being statistically significant (p-value > 0.05) due to the different analysis carried out for both kinds of data (*see Materials and methods – Data analysis*) and the few years available for calculating water deficit coefficients, which tends to increase standard errors.

Table 2: Results of the forward stepwise regressions between water deficit, aerobiological parameters and reproductive trees, mean mature acorn production per tree (Acorns) and annual interindividual variability coefficient (CVai) for *Q. ilex* and *Q. pubescens* and the two aerobiological stations. β ± SE are standardized partial beta coefficients. HDs: Spring water deficit, PMax: pollen production, DI: onset of flowering date, BCN: Barcelona and BTT: Bellaterra. R^2: Whole model goodness of fit. P-values: † < 0.1 * < 0.05 ** < 0.01 *** < 0.001.

a) *Quercus pubescens*		**β WDs**	**β DI**	**β PMax**	**R^2**
	Trees	-0.71 ± 0.22			0.50 *
	log Acorns	-0.57 ± 0.26			0.33 †
BCN	**CVai**	0.75 ± 0.21			0.56 **
	log Trees	-0.76 ± 0.21			57 **
	log Acorns	-0.61 ± 0.25			0.37 *
BTT	**log CVai**	0.70 ± 0.20 **	0.36 ± 0.20 †		0.66 **
b) *Quercus ilex*					
	log Trees	-0.60 ± 0.19*	0.47 ± 0.19*		0.68 **
	log Acorns	-0.52 ± 0.19 *	0.56 ± 0.19 *		0.67 **
BCN	**log CVai**	0.75 ± 0.23			0.56 *
	log Trees	-0.80 ± 0.16 ***	0.31 ± 0.16 †		0.76**
	log Acorns	-0.72 ± 0.16 **	0.44 ± 0.16 *	0.32 ± 0.16 †	0.80**
BTT	**CVai**	0.77 ± 0.18 **		-0.35 ± 0.18 †	0.72**

Table 3: Autocorrelation coefficients (mean ± SE) of acorn crop and water deficit of the Fabra observatory and Cerdanyola weather station. For the acorn production coefficients, standard errors and p-values are based on a t-test (N = 17). The standard errors of the water deficit are based on a white noise (independency) subjacent process while signification levels are based on Box Ljung statistic. ACF: Autocorrelation function; PACF: Partial autocorrelation function. P-values: † < 0.1 * < 0.05 ** < 0.01 *** < 0.001.

ACF	**lag 1**	**lag 2**	**lag 3**	**lag 4**	**lag 5**	**lag 6**
Acorns (*Q. pubescens*)	-0.21 ± 0.03***	-0.04 ± 0.04	-0.19 ± 0.03***	0.01 ± 0.04	-0.03 ± 0.04	0.17 ± 0.04**
Acorns (*Q. ilex*)	-0.23 ± 0.03***	0.02 ± 0.03	-0.23 ± 0.01***	-0.08 ± 0.03*	-0.06 ± 0.03*	0.32 ± 0.03***
WDs (Fabra)	-0.60 ± 0.26*	0.29 ± 0.24*	-0.15 ± 0.23†	-0.05 ± 0.22	-0.08 ± 0.20	0.10 ± 0.19
WDs (Cerdanyola)	-0.51 ± 0.26*	0.15 ± 0.24	-0.21 ± 0.23	0.11 ± 0.29	-0.14 ± 0.20	0.19 ± 0.19
PACF						
Acorns (*Q. pubescens*)	-0.21 ± 0.03***	-0.11 ± 0.03**	-0.22 ± 0.03***	-0.14 ± 0.05*	-0.14 ± 0.04**	0.01 ± 0.04
Acorns (*Q. ilex*)	-0.23 ± 0.03***	-0.04 ± 0.03	-0.24 ± 0.02***	-0.23 ± 0.03***	-0.22 ± 0.03***	0.16 ± 0.04**
WDs (Fabra)	-0.60 ± 0.29*	-0.11 ± 0.29	-0.04 ± 0.29	-0.22 ± 0.29	-0.35 ± 0.29	-0.17 ± 0.29
WDs (Cerdanyola)	-0.51 ± 0.29†	-0.14 ± 0.29	-0.27 ± 0.29	-0.16 ± 0.29	-0.25 ± 0.29	-0.05 ± 0.29

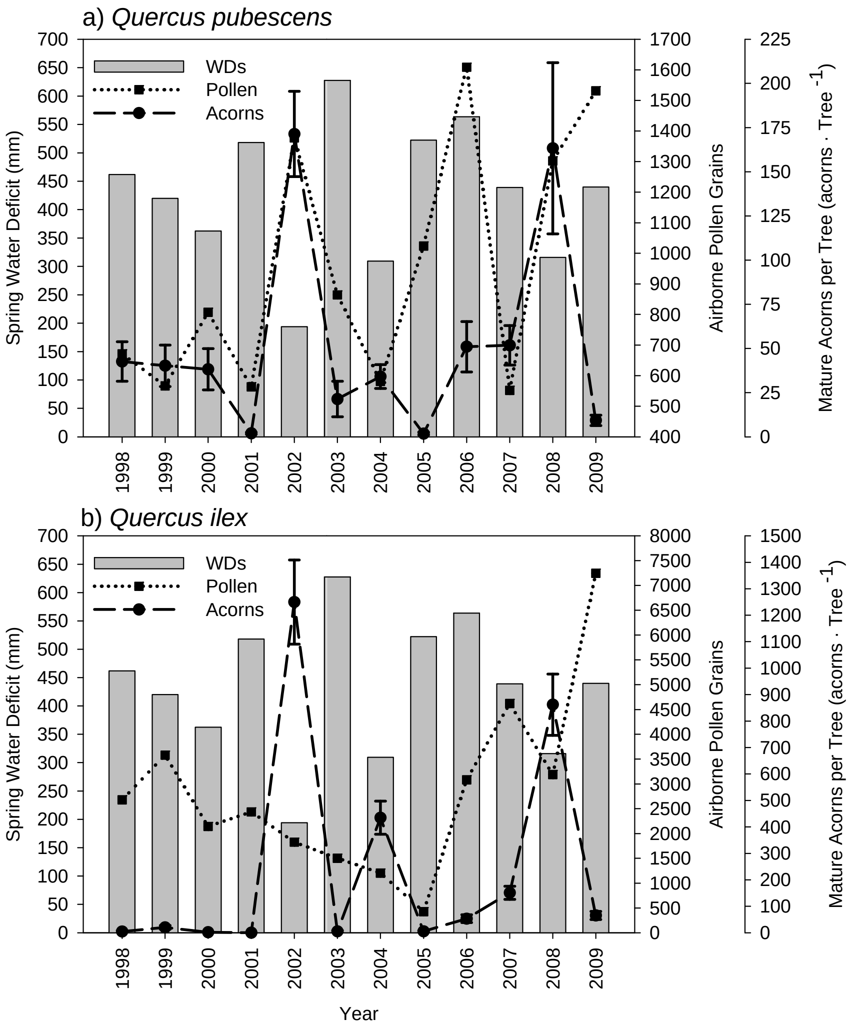

Figure 2:

Meteorological, aerobiological and acorn crop data of the study period (1998 – 2009). *a)* Water deficit, *b)* Airborne pollen captured until the peak (PMax) and *c)* mean mature acorns per tree (all plots included). Vertical bars show standard errors.

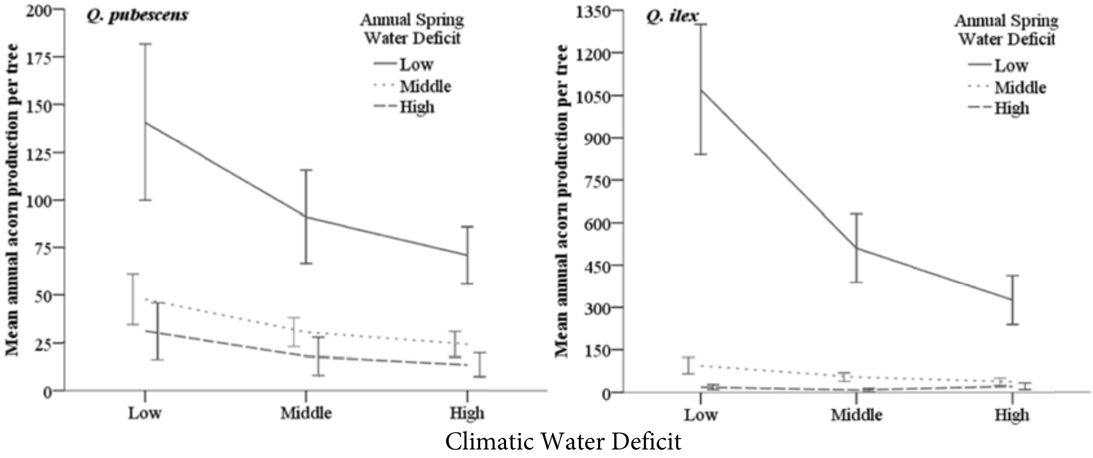

Figure 3: Interaction plot of the mean mature acorn crop per tree in relation to soil water availability (climatic water deficit) and annual spring water deficit for *Q. ilex* and *Q. pubescens*. Soil water availability and spring water deficit are presented categorized to provide an easier interpretation of the effects. Vertical bars show standard errors.

4. Discussion

This study provides two main contributions to understanding the masting behaviour of Mediterranean oaks. First, pollen availability was not the main cause determining either mature acorn crop or synchronization among individuals in comparison to the paramount effect of water deficit. This suggests that, in these forests, extreme interannual variability in acorn production and reproductive synchrony among trees seems to be mostly driven by *resource availability* and the *Moran effect* rather than *pollination efficiency* and *pollen coupling* (see also Espelta et al. 2008, Pérez-Ramos et al., 2010, Sánchez-Humanes and Espelta 2011). Nevertheless, we cannot completely rule out the contribution of other adaptive forces in shaping this reproductive behaviour (e.g. endogenous cycles related with *satiation and starvation of acorn predators*). Second, the date of the onset of flowering appeared as a relevant factor affecting acorn production in *Q. ilex*. To the best of our knowledge, this is the first time that a characteristic of flowering phenology has been observed to influence acorn production. Clearly, in the light of the predicted changes in plant phenology in Mediterranean areas driven by climate warming (Peñuelas et al., 2002; García-Mozo et al., 2006), this is a point that may require further attention. However, we must also take into account that our data set has limitations: the moderate length of data series (17 years for pollen records and 12 for acorn production) and the fact that the aerobiological stations were located relatively far away from the monitored forest sites. Therefore, further research is needed in order to support our conclusions.

Despite these limitations, our results prove that aerobiological records accurately reflect the flowering phenology of the adjacent forest sites. This supports the hypothesis that airborne pollen concentrations may be used as a proper proxy of the onset and the intensity of flowering (see also Jato et al., 2007). The two *Quercus* species included in this study presented different phenologies of flowering, with the winter-deciduous *Q. pubescens* blooming earlier than the evergreen *Q. ilex*. In any case, in the two oaks the onset of flowering and the amount of pollen released into the atmosphere were positively influenced by higher temperatures García-Mozo et al., 2002, 2006). Interestingly, the amount of rainfall and its temporal distribution barely influenced airborne pollen production, despite the importance that water scarcity has been suggested to have for biological processes in Mediterranean-type forests or the negative effects that excess rainfall may have on pollen emission (Knapp et al., 2001).

4.1. Drivers of masting: the role of airborne pollen

We did not find significant effects of airborne pollen load in the atmosphere either on the percentage of reproductive trees or on the acorn crop size per tree, in contrast with results obtained in other studies (see García-Mozo et al. 2007). This might be due to two causes. On the one hand, most studies which have claimed a relationship between airborne pollen load and acorn production (see Knapp et al., 2001; García-Mozo et al., 2007) have been done in savannah-like forests (with a tree density less than 100 trees ha^{-1}), where a great distance among neighbouring trees and a limited range of pollen spread (Sork et al., 2002) could make pollen availability a limiting factor for tree reproduction. In contrast, oak forests in our study area are characterized by a much higher density of trees (i.e. more than 1000 stems ha^{-1} in Collserola) as is the case for most oak forests in the rims of the Mediterranean Basin. On the other hand, as pointed out by Smith et al. (1990), the importance of irregular but extremely high airborne pollen loads to ensure pollination (the *pollination efficiency* hypothesis) should be more evident in species in which the costs of the production of female reproductive structures is high and similar, whether or not they are pollinated (e.g. cones in conifers). In contrast, non-pollinated female flowers or aborted acorns of oaks are dropped at an early stage, entailing scant wasting of resources (Shibata et al., 2002; Kelly and Sork, 2002, Espelta et al. 2009b). In addition, the fact that in our study area, pollen availability did not show any relationship with interindividual variability in acorn production, suggests that cues other than *pollen coupling* are the main factors enforcing tree synchrony in reproduction (e.g. temperature; Schauber et al., 2002, or drought; Wright et al. 1999 and Espelta et al. 2008).

4.2. Drivers of masting: the role of flowering phenology

Notwithstanding this lack of correlation between airborne pollen and seed production, both the number of trees producing acorns and mean acorn crop size per tree in *Q. ilex* presented a positive relationship with a later onset of flowering. This effect could be partly explained by the fact that in this perennial species, a later onset of flowering could allow some amount of early-spring photosynthates to be allocated to enhance the development of the floral buds initiated the preceding year. Indeed, although later onsets of flowering are driven by colder winters, it must be highlighted that photosynthesis activity in *Q. ilex* in our region may be very high in early spring (Sala 1999, Montserrat-Martí et al. 2009) when temperatures (11.8 ± 0.3 in March and 13.7± in April) are much higher than the threshold (below 6 ºC) reported by Gratani et al. (2000) below which photosynthetic rates in this species severely decreases. Conversely, the lack of an effect of the onset of flowering on acorn production in the winter-deciduous *Q. pubescens* could be due to the fact that in this winter-deciduous species catkins and leaves grow at the same time, and therefore a later onset of flowering does not ensure a potential surplus in photosynthates to be invested in flowering and acorn growth. In addition to this hypothesis, better environmental conditions in later spring could also be invoked when analysing the positive effect of the date of the onset of flowering in *Q. ilex*: i.e. the later the onset of flowering, the hotter the expected temperatures with a lower risk of cool and moist weather episodes that might wither catkins before pollen release (Knapp et al., 2001). However, the nonexistent relation between airborne pollen load and acorn production makes us discard this hypothesis.

4.3. Drivers of masting: the role of resource availability

In the two oaks, interannual differences in mature acorn crop and inter-individual synchrony were mostly prompted by spring water deficit, as previously predicted by other studies that did not consider pollen availability (Espelta et al., 2008; Pérez-Ramos et al., 2010). Yet the lack of a significant interaction between soil water availability and spring water deficit (i.e. spring water deficit has a similar negative effect in spite of the local water availability) and the absence of a relationship between population variability on acorn crop size per plot and soil water availability does not support the prediction that masting would be more severe in poorer sites as predicted in some previous studies (Kelly and Sork 2002, Espelta et al. 2008). However, it is possible that a wider range of variation in the soil water availability than the one covered in this study might be required to find this kind of relationships. The paramount importance of spring water deficit for acorn production supports the hypothesis of *resource matching* and the *Moran Effect* (Ranta et al., 1997) as the main drivers of masting (see Norton and Kelly, 1988; Sork et al., 1993, Isagi et al., 1997): i.e.

better spring conditions for photosynthesis resulted in higher acorn crops for both species. However, the negative effect on the current acorn crop size of the previous one (negative effect of lag -1 in the autocorrelation analyses) suggest also some effect of *resource accumulation*. Furthermore, *Q. ilex* presented a positive effect of the sixth lag's acorn production, inexistent neither for *Q. pubescens* as well as for meteorological data. This positive coefficient, coincident with the results reported by Pérez-Ramos et al. (2010,) in *Q. ilex* forests in Southern France suggests the existence of inherent cycles of producing mast crops (Sork et al., 1993) or endogenous cycles of storing and remobilization of resources (Pérez-Ramos et al., 2010; Sánchez-Humanes et al., 2011). Conversely *Q. pubescens* did not present any significant autocorrelation effect beyond that 1-year lag. This might be due to the fact that winter-deciduous species drop their leaves every year. This could hinder a long-term accumulation of resources if leaves acting as storage organs (Chabot and Hicks, 1982) or, as suggested by Camarero et al., (2010), masting is more correlated with leaf area accumulation rather than with an increase of biomass in perennial tissues (e.g. branches, trunk). Moreover, acorn production in *Q. pubescens* could be more constrained by *resource matching* (spring water deficit) than *Q. ilex*, due to the greater sensitivity to water stress of the former species (see Damesin et al. 1998), particularly in Collserola where *Q. pubescens* is near its southern distribution limit (Bolós and Vigo, 1990).

In light of the importance of interannual differences in water stress in spring to determine masting behaviour in Mediterranean oaks, there is great uncertainty as to how this reproductive behaviour will respond to climate change in this area. Several recent studies that have experimentally assessed the impact of new (drier) climatic scenarios on the reproduction of Mediterranean oaks have predicted a progressive decrease in acorn production and a greater inter-annual variability (see Ogaya and Peñuelas 2007, Sánchez-Humanes and Espelta 2011). Indeed, the more the spring temperatures will rise (IPCC, 2007), the more the water deficit will increase (see Thornthwaite, 1948). In addition, and according to our results, an earlier onset of flowering might additionally constrain acorn production in the perennial *Q. ilex*. Such a progressive reduction in acorn crop size and a higher interannual fluctuation may negatively impact the fauna directly feeding on this resource and the upper trophic levels (Cahill and Llimona, 2004). Therefore, further research is needed to accurately predict acorn production and to design new and appropriate forest management strategies to mitigate as much as possible the effects of climate change on the reproduction of Mediterranean oaks (Sánchez-Humanes and Espelta 2011).

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Chapter 8

Temporal trends in the enhanced vegetation index and spring weather predict seed production in Mediterranean oaks

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Abstract

The extremely year-to-year variable production of seeds (*masting*) is an extended plant reproductive behaviour important for forest dynamics and food webs. The dependence of these episodes of massive seed production on recently or long-term photosynthesised carbohydrates, however, remains controversial. In this paper, we explore whether vegetation (tree canopy) changes, detected using EVI as a proxy of leaf area and photosynthetic capacity, can provide a reliable estimation of seed production. To complete this analysis, we also explored the effect of weather both in the trends of EVI and in acorn crop size. To this end, we compared the trends of the EVI and acorn production over 10 years (2000-2009) in five stands of *Quercus ilex* L. in Barcelona (Catalonia, NE Spain). We found that acorn production was mainly driven by a combination of: i) a minimum initial threshold in the EVI values, ii) an increase in EVI in the 9 ± 4 months prior to reproduction, and iii) appropriate weather conditions (low water stress) during spring. These results indicated, apparently for the first time, that reproduction in masting species could be detected and partly predicted by remotely sensed vegetative indices. Our results suggested that this particular reproductive behaviour in Mediterranean oaks was driven by a combination of two factors, i.e. good and improving vegetation conditions, as shown by a minimum initial threshold and the increase in EVI needed for large seed crops, and the need of wet weather conditions during spring. Moreover, our results fully supported recent studies that have associated short-term photosynthate production with seed production.

1. Introduction

The synchronous but erratic year-to-year production of seeds, i.e. *masting*, is a widely geographically and taxonomically extended reproductive behaviour (Kelly and Sork 2002). Masting events have cascading effects on several forest functions and processes (Ostfeld and Keesing 2000) such as seedling establishment (Negi et al. 1996, Espelta et al. 2009), the regulation of the populations of seed consumers (McShea 2000, Espelta et al. 2008), and the propagation of diseases (e.g. lyme disease (Ostfeld, 1997)). Notwithstanding this, what drives this phenomenon is one of the most puzzling questions about the dynamics of forest ecosystems (Koenig and Knops 2005).

Whether seed production in masting species is controlled by recently acquired or accumulated resources (e.g. carbohydrates and nutrients) remains controversial (Koenig and Knops 2000; Kelly and Sork 2002; Sala et al. 2012). On the one hand, the *resource matching* hypothesis (Sork et al. 1993; Kelly and Sork 2002) states that plants produce large or poor seed crops in direct response to the immediate availability of resources, i.e. more favourable weather conditions for photosynthesis would lead to larger harvests (e.g. Espelta et al. 2008; Espelta et al. 2009). On the other hand, the *resource budget model*, or the *resource accumulation* hypothesis (Sork et al. 1993; Isagi et al. 1997), suggests that plants produce extraordinary seed crops when a resource threshold is reached after several years of progressive accumulation. This latter hypothesis predicts that plant resources are depleted to sustain the masting episode, so that flowering and seed production are expected to be very low or absent in the next growing season, causing a negative autocorrelation between consecutive seed crops (Koenig and Knops 2000). The consequences of resource depletion after a masting episode may include signs such as a reduction in the leaf area due to self-thinning (Camarero et al. 2010) or a decrease of nitrogen and phosphorous concentrations in branches (Sala et al. 2012).

Recent studies based on carbon isotopes suggest that seed production in several tree species in temperate forests depends only on the carbohydrates photosynthesised during the months prior to seeding (Ichie et al. 2013; Hoch et al. 2013). Unfortunately, the measurement of resource availability at a plant level is time-consuming and expensive and so is difficult to perform at broad spatial and temporal scales. Conversely, remotely sensed vegetation indices may be useful proxies for monitoring the fluctuations of trees resources at large temporal and spatial scales. Indeed, spectral indices such as the Normalized Difference Vegetation Index (NDVI) and the Enhanced Vegetation Index (EVI) provide good descriptions of the state of green vegetation (e.g. crown cover, leaf area index, and greenness) and its temporal dynamics. These indices have thus been widely used to monitor the functional traits involved in the carbon cycle (Garbulsky et al. 2008) and also as annual integrators of carbon uptake in forests (Garbulsky et al. 2013). Few studies, however, have yet applied remotely sensed vegetation indices to predict seed crop production in forest ecosystems, particularly in masting species (but see Camarero et al., 2010). In this sense, a successful methodology to predict seed crops would allow managers to better plan management actions in the near future (e.g., wildlife conservation strategies).

The aim of this study was to explore whether the pattern of seed production in Mediterranean oak (*Quercus* spp.) forests could be governed by the temporal dynamics of tree canopies, assessed by means of remote sensing indices. We hypothesise that vegetation changes, detected using EVI, represent a reliable proxy of the tree resources available for seed production. Therefore, if an accumulation of resources (e.g. carbohydrates and nutrients) for several years is a prerequisite for a masting event, EVI should progressively increase during that period of accumulation. Similarly, if a severe depletion of resources occurs after a masting episode, EVI should decrease due to the self-thinning of the canopy. In addition, given the nature of the Mediterranean climate, we hypothesise that weather conditions (e.g. drought) must be an important driver of the temporal dynamics of both EVI and acorn crop size. To address these questions, we used a data set comprising 12 years (1998-2009) of acorn production in five oak stands in Collserola Natural Park (Catalonia, NE Spain) and 11 years (2000-2010) of EVI data derived from MODIS (MODerate Resolution Imaging Spectroradiometer) images of each forest stand.

2. Materials and methods

2.1. Study area and species

This study was conducted in the Collserola massif (41º26′N, 02º06′E), whose vegetation is dominated by *Q. ilex*. The study area has a Mediterranean climate and a mean annual temperature of 15.7 ± 1.4 ºC. January is the coldest month (8.5 ± 0.6 ºC) and August the warmest (24.3 ± 0.7 ºC). Mean annual precipitation reaches 613.8 ± 34.0 mm (coefficient of variation (CV) = 0.25; values from the Fabra Observatory at 415 m.a.s.l. for the current climatological standard normal period, 1991-2010). We selected forest stands of *Q. ilex* because it is the most abundant tree species in Collserola, has a strong masting behaviour (Espelta et al. 2008; Fernández-Martínez et al. 2012), and has evergreen leaves that allow continuous monitoring using remotely sensed vegetative indices. Regarding *Q. ilex* fruiting phenology, the pollination period normally starts during the first week of April and pollen release reaches its maximum during May (Fernández-Martínez et al. 2012). Pollinated flowers are then ripened until mid-September, when the harvest begins. We selected five 10 m radius stands (plots A to E), distributed along the Collserola massif, of nearly pure *Q. ilex* stands to maximise the accuracy of estimates of leaf area (more than 72% of the trees were *Q. ilex* in all plots) being representative of the nearby area. The selected forests were very dense (coppiced stands of 1357 ± 219 *Q. ilex* stems per hectare), mostly resulting from resprouting and relatively young (ca. 40-60 years), presenting 10.5 ± 0.4 cm as mean diameter at breast height and around 3 to 4 m of height.

2.2. Experimental design and sampling

2.2.1. Acorn crop data

Acorn production was monitored from 1998 to 2009. Fifteen trees per plot were tagged, and the number of branches per tree was estimated using a regression model between crown projection and the number of branches previously determined for a subsample of trees (see Espelta et al. 2008). Acorn production on four branches per tree was recorded each September, i.e. at the peak of seed production in holm oaks before acorns were ripe enough to fall or to be harvested in appreciable number by birds (see Abrahamson and Layne 2002). The total number of acorns produced per tree was estimated by multiplying the mean acorn production per branch by the number of branches per tree (see Espelta et al. 2008 for further details). Since aborted and infested seeds usually fall much before maturation happens, insect-infested or aborted acorns do only consume a small amount of the tree resources, mostly insignificant in comparison with those needed in a mast year. Therefore, we did not take these acorns into account.

2.2.2. Estimates of canopy cover: satellite data

The NDVI and the EVI can be used to monitor temporal and spatial changes in leaf area and in the fraction of photosynthetically active radiation (fPAR) intercepted by the canopy (Myneni et al. 2002; Fensholt et al. 2004; Sprintsin et al. 2007). Both indices provide similar information, but we decided to use the EVI because: i) the NDVI saturates earlier at high levels of greenness (Huete et al. 2002) and our forests were very dense and ii) because it has been recently demonstrated to successfully monitor biomass in Mediterranean holm oak (Ogaya et al. 2015). We thus used the EVI values as surrogates of photosynthesising leaf area and the available resources of the trees. Data were extracted from the Terra MOD13Q1 product of MODIS for the period between 18 February 2000 and 19 December 2010, with a 16-day periodicity and a spatial resolution of 250 x 250 m^2 (pixel size = 6.25 ha), for the pixels that covered each forest stand. The pixels selected were almost entirely homogeneous with regard to vegetation type and crown cover, thus coinciding with the features of the sampled trees.

2.2.3. Meteorological data

Acorn crop size in Collserola is highly controlled by weather conditions (Fernández-Martínez et al. 2012). We thus gathered meteorological data from the Fabra Observatory (41°25′N, 02°07′E) to calculate the Standardized Precipitation-Evapotranspiration Index (SPEI, Vicente-Serrano et al. 2010) on a monthly basis and for the whole time series for time scales of 1, 3, 6, 9, and 12 months using the SPEI package (Begueria and Vicente-Serrano 2012) of R (R Core Team 2013). Positive values of the SPEI indicate above-average wet periods, and negative values indicate drought. Since vegetation may present a lagged responses to prolonged drought periods (Vicente-Serrano et al. 2013) SPEI was calculated for different time scales to determine the influence of weather conditions on acorn production and the EVI signal for different periods of time.

2.3. Data analysis

We first calculated mean acorn production per tree for each plot and year. Then we described the masting behaviour of our holm oak populations using the most common metrics (Kelly and Sork 2002): i) the CVs of seed production as measures of interannual variability at the population (CV_p) and the individual (CV_i) levels, ii) Spearman's *r* coefficients as measures of synchrony in seed production among plots (r_p) and among individual trees within a plot (r_i), and iii) the mean temporal autocorrelation of seed production of the trees within a plot using simple and partial autocorrelation functions (ACF and PACF, respectively) to the fourth time lag. We also calculated these metrics for the mean annual EVI values to check for similarities in the time series of both acorn production and the EVI. Standard errors of these variables were calculated using the bootstrap method (Efron 1987).

2.3.1. Testing the effect of weather on EVI time series

We tested the effect of weather on the EVI by two methods. i) We first calculated the standardised and deseasonalised monthly EVI time series using an additive seasonal decomposition. These time series indicated whether a particular month (e.g. January 2000) was above or below the mean for that month (January) in units of standard deviations (SDs). We then smoothed the EVI time series using local regressions (LOESS, Cleveland 1979) to remove noise and identify trends or low-frequency periodicities in the time series. This methodology would also help to deal with potential noise addition in the estimation of EVI as a consequence of the flowering intensity (Shen et al. 2014). Finally, we correlated the smoothed EVI time series with the SPEI time series (1, 3, 6, 9, and 12) using Pearson correlations. ii) We calculated the seasonally averaged EVI and SPEI values (e.g. winter EVI: mean EVI from January to March) for each year to correlate with each other (EVI vs.

SPEI) to identify the season in which the SPEI was better correlated with the EVI. Spearman correlations were used for this procedure. To avoid increasing false discovery rate, *P* values were adjusted using the Bonferroni correction.

2.3.2. Testing the effect of weather and the EVI on seed production

We performed a two-step analysis to determine the relationships among weather, the EVI, and acorn production. First, we explored the relationships of seasonally averaged SPEI and EVI time series with acorn production to identify the season when SPEI has the largest effect on EVI. Then, we tested the correlation between acorn production and the seasonally averaged time series of SPEI and EVI. We used Spearman correlations for this procedure because the acorn-production data were not normally distributed. Second, we used the smoothed EVI time series to test whether tree resources increased before large seed crops (i.e. masting events) by looking for evidence of an increasing EVI prior to masting. We identified all changes in the trends (e.g. from increasing to decreasing EVI) of the smoothed time series and considered an EVI trend to be the period between two of these points of change (peaks or valleys). We chose not to calculate trends for fixed periods of time because weather drives the phenology of these trees from year to year (Fernández-Martínez et al. 2012), and the periods of resource accumulation might thus occur during slightly different periods depending on the particular meteorological characteristics of the year. Then we assigned each trend to the most plausibly influenced harvest, starting at least five months (spring) before the harvest season (October). We also calculated the initial and final EVI values (EVI_i and EVI_f, respectively, units in SD) and the increment in the EVI (i.e. $\Delta EVI = EVI_f - EVI_i$) of each trend.

To test the influence of weather, the EVI, and previous acorn harvests on acorn production, we constructed a generalised linear mixed model (GLMM) fitted using the negative binomial distribution, using the natural logarithm as the link function, to correct for overdispersion. The model included the plot as a five-level random factor. The fixed effects were an SPEI variable (best correlated with acorn production), ΔEVI, EVI_i, EVI_f, and the acorn production of the previous year as covariates. We also included the interaction of ΔEVI with EVI_i and EVI_f to test for possible synergic effects of an increasing EVI and the initial or final level reached in the EVI. The inclusion of the acorn production of the previous year allows the testing for a negative autocorrelation in seed production that would suggest a depletion of resources after mast years (Sork et al. 1993). The minimum adequate model was achieved by deleting all non-significant predictors and minimising the Akaike information criterion. The models were fitted using the R package *lme4* (Bates et al. 2013).

2.3.4. Testing for the reduction of leaf area after large seed crops

To test whether the crowns of trees were self-thinned after mast seeding, we analysed the entire 16-day EVI time series with the Breaks For Additive Seasonal and Trend (BFAST) methodology using the R package *bfast* (Verbesselt et al. 2010a; Verbesselt et al. 2010b). We used this technique due to its ability to integrate the decomposition of time series into trend, seasonal, and residual components with methods for detecting changes in trends and seasonal behaviour. We configured BFAST to detect one break per year because we expected to find breaking points in the deseasonalised EVI time series after episodes of mast seeding.

3. Results

3.1. Synchrony, interannual variability, and temporal autocorrelation in acorn production and the EVI

Two clear mast seeding episodes occurred in our experimental plots, in 2002 and 2008 (Figures 8.1 and 8.2). Another masting event of extremely high acorn production occurred in plot D in 2004, but the other stands produced moderate amounts of acorns, so that we could not clearly define 2004 as a mast year for our holm oak plots (Figure 8.2). The time series of acorn production in all forest stands, however, presented a typical masting profile: high synchrony (r_i among trees and r_p among plots), high interannual variability at the individual (CV_i) and the population (CV_p) levels, and negative temporal autocorrelation (Table 8.1). No significant differences were found for r_p, r_i, CV_p, CV_i, and temporal autocorrelation among plots (using bootstrapped standard errors of the means). The average synchrony (r_p) in the mean annual EVI was 0.68 ± 0.03 (Table 8.1). The CVs of the EVI values were very low (~0.05) and did not differ from zero (were not significant) due to error propagation, although the *r* values were quite high.

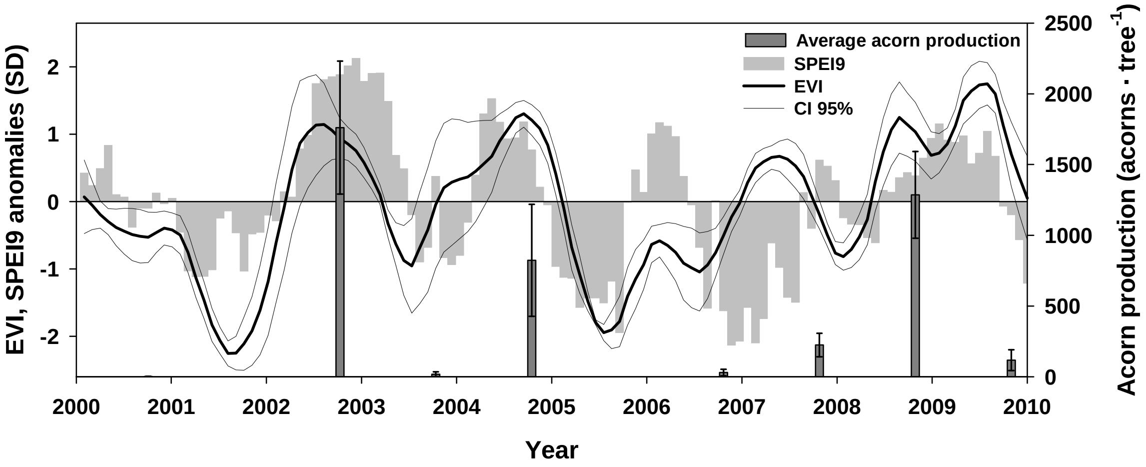

Figure 8.1. Smoothed and deseasonalised anomalies of EVI time series averaged for all five plots overlaid to SPEI9. Humid periods (positive SPEI9) lead to positive anomalies in the EVI ($r = 0.50$, $P < 0.001$). Grey bars indicate the average annual acorn production of the five plots. Error bars indicate standard errors. SD, standard deviation; CI, confidence interval.

Table 8.1. Synchrony (r_p among plots and r_i among trees), variability at the population (CV_p) and individual (CV_i) levels, and temporal autocorrelation (simple and partial (P)) of acorn production and EVI time series (mean ± SE). No significant differences were found among plots. Values different from zero are in bold type.

	Plot	r_p	r_i	CV_p	CV_i	lag 1	lag 2	lag 3	lag 4	lag 2 (P)	lag 3 (P)	lag 4 (P)
Acorns	A	**0.89 ± 0.26**	**0.64 ± 0.08**	**1.74 ± 0.42**	**2.26 ± 0.14**	**-0.16 ± 0.03**	-0.05 ± 0.03	**-0.17 ± 0.02**	**-0.09 ± 0.02**	**-0.09 ± 0.03**	**-0.19 ± 0.02**	**-0.20 ± 0.03**
	B	**0.85 ± 0.29**	**0.69 ± 0.10**	**1.86 ± 0.49**	**2.17 ± 0.12**	**-0.24 ± 0.02**	0.03 ± 0.04	**-0.15 ± 0.03**	**-0.17 ± 0.03**	-0.03 ± 0.04	**-0.16 ± 0.04**	**-0.29 ± 0.03**
	C	**0.91 ± 0.21**	**0.62 ± 0.06**	**1.77 ± 0.48**	**2.27 ± 0.13**	**-0.20 ± 0.02**	0.02 ± 0.04	**-0.17 ± 0.02**	**-0.11 ± 0.04**	-0.03 ± 0.04	**-0.18 ± 0.01**	**-0.22 ± 0.05**
	D	**0.84 ± 0.28**	**0.66 ± 0.06**	**1.61 ± 0.47**	**2.06 ± 0.17**	**-0.20 ± 0.02**	0.02 ± 0.04	**-0.17 ± 0.02**	**-0.11 ± 0.04**	-0.03 ± 0.04	**-0.18 ± 0.01**	**-0.22 ± 0.05**
	E	**0.89 ± 0.25**	**0.73 ± 0.10**	**2.03 ± 0.52**	**2.12 ± 0.14**	**-0.16 ± 0.04**	0.00 ± 0.04	**-0.18 ± 0.02**	-0.06 ± 0.03	-0.05 ± 0.03	**-0.18 ± 0.03**	**-0.17 ± 0.04**
	mean	**0.87 ± 0.01**	**0.65 ± 0.02**	**1.80 ± 0.07**	**2.18 ± 0.04**	**-0.19 ± 0.01**	0.00 ± 0.01	**-0.17 ± 0.01**	**-0.11 ± 0.02**	**-0.05 ± 0.01**	**-0.18 ± 0.01**	**-0.22 ± 0.02**
EVI	A	0.70 ± 0.41		**0.05 ± 0.01**		0.32 ± 0.30	-0.04 ± 0.33	-0.20 ± 0.33	-0.32 ± 0.34	-0.16 ± 0.30	-0.15 ± 0.30	-0.24 ± 0.30
	B	0.73 ± 0.49		**0.05 ± 0.01**		0.22 ± 0.30	-0.25 ± 0.32	-0.18 ± 0.33	-0.05 ± 0.34	-0.31 ± 0.30	-0.05 ± 0.30	-0.08 ± 0.30
	C	0.57 ± 0.58		**0.04 ± 0.01**		0.06 ± 0.30	0.08 ± 0.30	-0.39 ± 0.31	-0.06 ± 0.35	0.08 ± 0.30	-0.40 ± 0.30	-0.01 ± 0.30
	D	0.76 ± 0.42		**0.05 ± 0.01**		0.32 ± 0.30	-0.15 ± 0.33	-0.04 ± 0.34	0.00 ± 0.34	-0.29 ± 0.30	0.14 ± 0.30	-0.10 ± 0.30
	E	0.64 ± 0.55		**0.04 ± 0.01**		0.17 ± 0.30	-0.41 ± 0.31	-0.48 ± 0.36	0.00 ± 0.41	-0.45 ± 0.30	-0.39 ± 0.30	-0.06 ± 0.30
	mean	**0.68 ± 0.03**		**0.05 ± 0.01**		**0.22 ± 0.05**	**-0.16 ± 0.08**	**-0.26 ± 0.08**	**-0.09 ± 0.06**	**-0.23 ± 0.09**	-0.17 ± 0.10	**-0.10 ± 0.04**

3.2. Effects of weather and an increasing EVI on acorn production

Droughts and wet periods (i.e. negative and positive SPEI9 values – SPEI for a 9-month window) were correlated with low and above-average EVI values, respectively (Figure 8.1). The correlation analysis confirmed that the EVI could be driven by the cumulative effect of weather conditions during the previous nine months ($r = 0.50$, $P < 0.001$; Table 8.3). Additionally, acorn crops were larger after wetter seasons and when they were preceded by larger increases in the EVI during the previous months (Figures 8.1 and 8.2). Acorn crop sizes, however, were still very low when these periods of increasing EVI began from very low EVI values and did not reach above-average values (Figures 8.1 and 8.2).

The selected periods of increasing or decreasing EVI values (Figure 8.2, shaded periods) lasted, on average, 9 ± 4 months (range: 4-16 months). Most of these periods began during the summer or autumn of the previous year or during the winter of the year in which acorns were produced, and they ended mostly in summer (Figure 8.2). Acorn crop size was positively correlated with $\mathrm{SPEI6}_{\mathrm{Jn}}$, $\mathrm{EVI}_{\mathrm{i}}$, and $\Delta\mathrm{EVI}$ (Table 8.2). The best model predicting acorn production included $\mathrm{SPEI6}_{\mathrm{Jn}}$ (i.e. SPEI comprising six months from January to June), $\mathrm{EVI}_{\mathrm{i}}$, $\Delta\mathrm{EVI}$, and a positive interaction between $\mathrm{EVI}_{\mathrm{i}}$ and $\Delta\mathrm{EVI}$. In this model, the effect of acorn production in the previous years was negligible. These results confirmed the visual inspection of Figures 8.1 and 8.2: large acorn crops were correlated with both wet spring periods and a previous trend of increasing EVI but depended on the values at which this trend started. Interestingly, the positive interaction between $\mathrm{EVI}_{\mathrm{i}}$ and $\Delta\mathrm{EVI}$ on acorn production (Table 8.2; Figure 8.3) highlighted the importance of reaching an initial EVI threshold before the beginning of the increasing EVI period.

Table 8.2: Output of the generalised linear mixed model using a negative binomial distribution (theta = 0.4013) relating acorn crop size, $SPEI6_{Jn}$, EVI_i, and ΔEVI, with plot as a random effect. SD, standard deviation; SE, standard error.

Random effects

Groups	Name	Variance	SD
Plot	Intercept	0.1203	0.3469
Residual		0.7708	0.8779

Fixed effects

	Estimate	SE	t value	P value
Intercept	4.31	0.30	14.48	<0.0001
ΔEVI	1.12	0.26	4.38	<0.0001
EVI_i	-0.47	0.41	-1.15	0.2515
$SPEI6_{Jn}$	1.01	0.46	2.20	0.0278
$\Delta EVI^{*} EVI_i$	0.45	0.13	3.52	0.0004

Table 8.3. Correlation coefficients for the SPEI9 and EVI time series, annual acorn production (AP), SPEI6 from June ($SPEI6_{Jn}$), and summer EVI (EVI_{sm}). Pearson correlations were used for EVI vs. SPEI9 and EVI_{sm} vs. $SPEI6_{Jn}$, and Spearman correlations were used for the AP relationships (due to the non-normal distribution of the data). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Plot	EVI vs. SPEI9	AP vs. EVI_{sm}	AP vs. $SPEI6_{Jn}$	EVI_{sm} vs. $SPEI6_{Jn}$
A	0.48^{***}	0.69^{*}	0.68^{*}	0.74^{**}
B	0.42^{***}	0.85^{**}	0.82^{**}	0.81^{**}
C	0.41^{***}	0.94^{***}	0.74^{*}	0.70^{*}
D	0.36^{***}	0.81^{**}	0.82^{**}	0.72^{*}
E	0.50^{***}	0.75^{*}	0.75^{*}	0.94^{***}

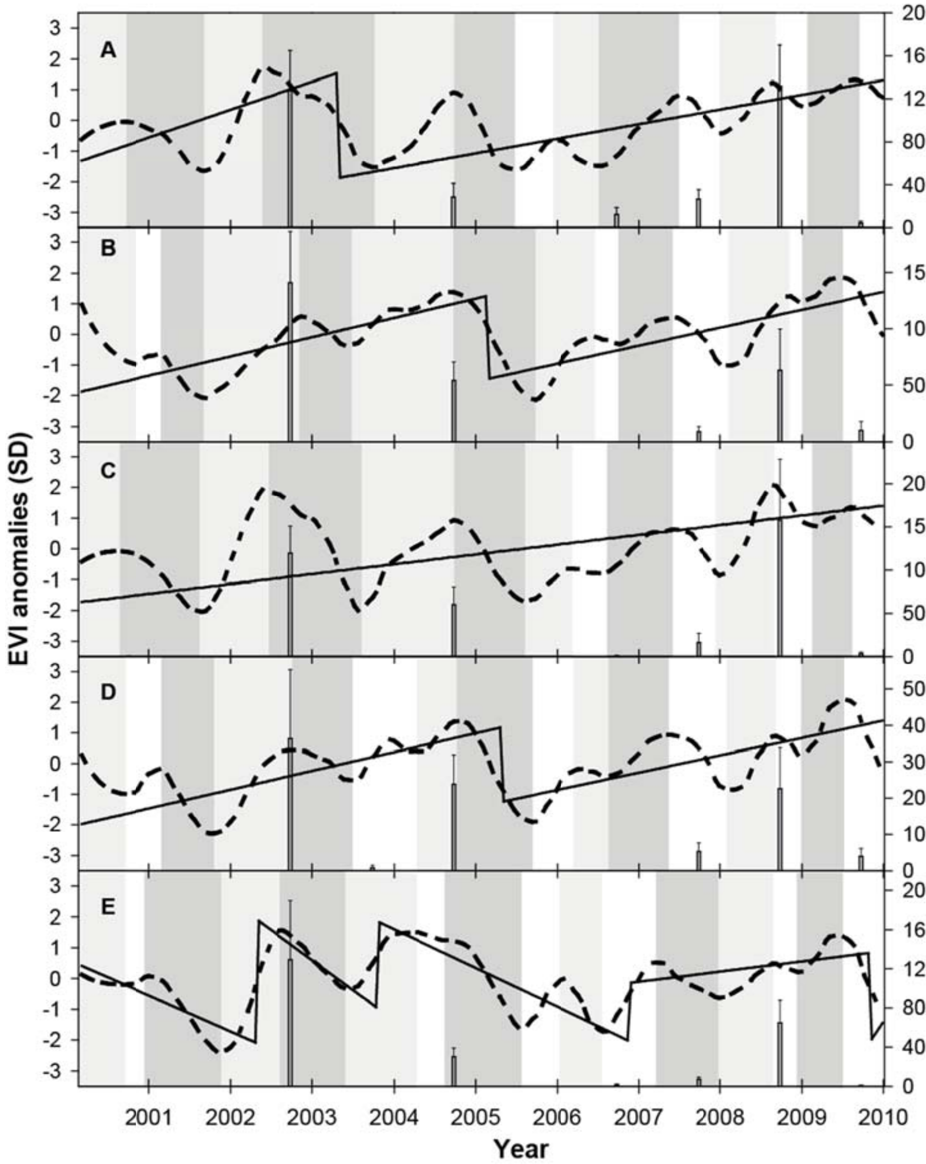

Figure 8.2. Temporal dynamics of the EVI time-series anomalies, BFAST trends identified in the EVI time series, and acorn production from 2000 to 2010 for each forest stand (A-E). Dashed lines represent the deseasonalised, smoothed EVI anomalies, solid lines show the BFAST trends of the series, and bars indicate annual acorn production. Light and dark shading indicate the periods used to calculate ΔEVI. Unshaded periods were not used to calculate these increments (see section 2.3.2 for information about the selection of periods). Error bars indicate standard errors.

3.3. Effects of masting events on crown condition

The BFAST methodology failed to identify a clear pattern of abrupt decreases in the trend of the EVI after the two observed masting events (Figure 8.2). Two stands (B and D) presented a break during 2005, and one plot (A) presented a break after 2003, possibly associated with the severe droughts that occurred in those years. Another plot (C) did not present any breaks during any time series, and only two of the five plots (A and E) presented one break that could be associated with the masting episode of 2002.

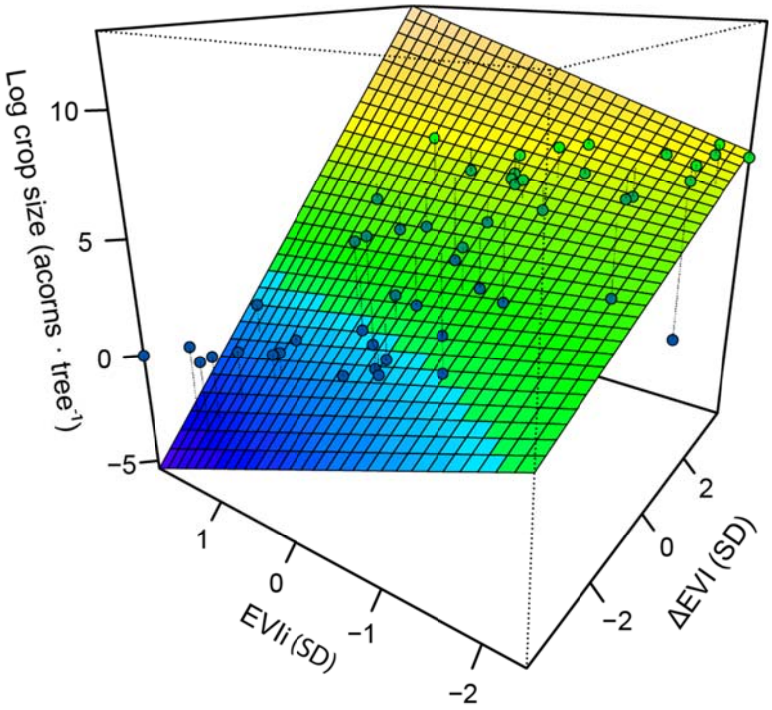

Figure 8.3: 3D representation of the response surface of acorn production to changes in ΔEVI and EVI_i. SD, standard deviation.

4. Discussion

To our knowledge, we report for the first time that mast seeding events can be detected and, to some extent, predicted using remotely sensed vegetative indices such as the EVI. The masting events observed in this study followed an average period of increase in the EVI (a surrogate of potential photosynthetic capacity) of 9 ± 4 months but also required favourable weather conditions in spring (low levels of water stress, $SPEI6_{Jn}$). In addition to these two factors, the initial EVI value at the start of an EVI increase period was also important, i.e. a large increase in the EVI did not lead to a masting event unless it departed from a minimum EVI_i threshold (Table 8.2; Figures 8.1, 8.2, and 8.3). This initial EVI threshold may suggest the need of a minimum amount of previously stored resources to produce buds before a mast seeding event, while the importance of the seasonal increase in the EVI and the favourable weather conditions in spring supports the relevance of the immediate availability of resources for triggering the masting episode. Our results thus fully agree with those recently found for deciduous trees in studies that applied isotopic analyses to show that the main photosynthates spent in the production of seed crops were those accumulated during the last growing seasons (within 17 months) (Ichie et al. 2013; Hoch et al. 2013).

4.1 Short-time resource accumulation and masting

The need of a minimum threshold of the EVI to produce large seed crops at the beginning of the increasing or decreasing EVI periods, despite the increase in the EVI during the last months before reproduction, supports the hypothesis that trees require a minimum level of stored resources to produce buds that will eventually ripen and produce seeds (Sork et al. 1993; Isagi et al. 1997; Kelly and Sork 2002). This minimum EVI value is in line with many studies reporting a relevant role of the conditions of the previous years (e.g. canopy growth, bud development, and temperatures) to account for a future masting episode (Alla et al., 2011, Sánchez-Humanes et al. 2011, Kelly et al., 2013). Moreover, the negative autocorrelation coefficients among consecutive acorn crops (Table 8.1, lags 1, 3, and 4) also support the premise that large crop sizes are driven by endogenous cycles of resource accumulation-depletion. When taking into account the EVI temporal dynamics and SPEI, however, the negative autocorrelation at lag 1 was not significant (Table 8.2), which indicates the relatively small importance of these endogenous cycles in comparison with the paramount role of the current year's weather conditions. The BFAST analyses, in contrast to Camarero et al. (2010), did not indicate that leaf area decreased due to crown self-thinning after a masting episode. The lack of such an effect in our oak stands could be due to several reasons: i) the low number of acorns produced in these moderately young forests (coppiced stands of ca. 40-60 years; see Espelta et al. 2008; Espelta et al. 2009) relative to the number produced in older forests, ii) oaks in the mild Mediterranean climate of our study area can even photosynthesise in autumn and early winter after the seeding season (Sánchez-Humanes 2009; Ogaya et al. 2014), thus replenishing a certain amount of the resources spent in reproduction and allowing to preserve more leaf area, and iii) the frequent droughts in our study area (see the values for SPEI9 in Figure 8.1) may be a much more relevant factor accounting for the changes in leaf area than the amount of resources invested in acorn production.

Once a minimum threshold of EVI was reached, the increase in EVI during the months (9 ± 4) previous to reproduction also influenced acorn production. The relationship between the leaf area index and gross primary production is well established (Gower et al. 2001; Law et al. 2002; Fernández-Martínez et al. 2014), and the EVI is proficient at estimating forest growth in Mediterranean evergreen forests (Garbulsky et al. 2013), but we cannot determine the part of the increase in the EVI that corresponds to an increase in leaf area or photosynthesis. The increase in leaf area during spring leads to higher rates of carbon fixation, thus generating the pool of carbohydrates needed to sustain a mast seeding event (Figures 8.1 and 8.2). These recently synthesised carbohydrates could be those identified in isotopic studies (Ichie et al. 2013; Hoch et al. 2013). However, since only deciduous species were used in these studies, caution should be taken when extrapolating these conclusions for the evergreen *Q. ilex*. The relatively short time periods of increasing EVI values (i.e. acquisition of resources) mediated by favourable weather conditions (e.g. low water stress) could be a consequence of drought episodes (see Figure 8.1, negative SPEI9 values for years 2005 and 2007) that may often interrupt a more constant accumulation of resources through the years. These results are consistent with the nature of the Mediterranean climate in which the growth of vegetation is mostly limited by severe shortages of water (Kramer et al. 2000).

The effect of weather on Mediterranean forests, however, may not only be of relevance for water availability. Precipitation may also increase mineralisation rates in soils (Jarvis et al. 2007) and nutrient availability to plants (Sardans and Peñuelas 2007; Smaill et al. 2011) in Mediterranean areas. The combined pulse of water and nutrient availability may thus produce a synergic effect boosting large seed crops in these forests. Accordingly, the reproductive behaviour of *Q. ilex* would be mostly aligned with that of a *putative* masting species that mimics the behaviour of the weather (*sensu* Koenig and Knops, 2000).

4.2 Rethinking the influence of weather on masting behaviour

Previous studies have suggested that weather could rarely be the most important factor driving masting due to the bimodality usually presented by seed production (Norton and Kelly 1988; Herrera et al. 1998), given the absence of bimodality in weather variables, and due to the higher variability of seed production compared to the variability of weather variables such as rainfall (Kelly and Sork 2002; Koenig and Knops 2005). These assumptions are unquestionably true for linear relationships between weather and seed production but not for non-linear relationships, as a consequence of *Jensen's inequality* (Jensen 1905; Ruel and Ayres 1999). The key role of weather found in this study, presenting a logarithmic relationship with acorn production, suggests that non-linear relationships between weather and seed production might be the cause of these differences in the distribution of seed production and weather data. For example, if seed production can be modelled as an exponential function of rainfall, the variability in seed production will easily be much higher than the variability in rainfall. Similarly, a sigmoid relationship between weather and seed production would lead that bimodality to appear in the data for seed production (e.g. flowering intensity of *Chionochloa* sp. in McKone et al. (1998)). The *Moran Effect* or synchronisation using weather cues (Ranta et al. 1997; Kelly et al. 2013) could also account for this non-linear effect of weather variability and seed production. These exosomatic signals may act as triggers for mobilising stored reserves in plants to produce seeds. We do not suggest (or report) that weather is the only driver of seed production and its extreme variability, but we call for a renewed view of the effect of weather and a reinforcement of its influence in conditioning masting behaviour either as a cue

(Kelly et al. 2013) or as providing good conditions for photosynthesis (Fernández-Martínez et al. 2012).

4.3 Are remotely sensed vegetative indices suiTable 8.for monitoring masting?

Our results clearly indicate that mast seeding events in *Q. ilex* can be detected by the analysis of trends in remotely sensed vegetative indices such as the EVI. According to our results, large seed crops in *Q. ilex* might be anticipated when detecting large increasing trends in the deseasonalised EVI, mostly since the winter prior to seed harvest, especially if the trend begins at high EVI values and weather conditions during spring are mostly wet. Other authors have suggested that remotely sensed vegetative indices could predict yields in annual agricultural crops (Zhao et al. 2007), but to our knowledge, this study is the first to demonstrate that seed production in forest ecosystems can also be detected using satellite imagery and weather data. Nevertheless, the study of species with different cycles of flowering, pollination, and maturation of seeds (e.g. red oaks that require more than one year from the onset of flowering to seed maturation), may require a different approach. Likewise, the study of deciduous forests may also require different methodologies, because these species may respond differently. For example, a drastic reduction in crown cover was detected in two birch species (*Betula alleghaniensis* and *B. papyrifera*) during a mast year (Gross 1972), and *Fagus sp.* has also shown drastic reductions in crown conditions due to large seed harvests (Innes 1992; Ferretti et al. 1998; Han et al. 2008; Vicca et al. 2014). In these kind of forests, masting could be detected after substantial decreases in the EVI not explained by weather conditions (Vicca et al. 2014). Various approaches may thus be needed to monitor and predict seed production in different plant species according to their contrasting functional and life-history traits. Establishing a reliable methodology for predicting masting events in forest ecosystems for extensive and remote areas will help to improve pest controls, wildlife management and hunting policies.

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