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http://hdl.handle.net/10026.1/1598

http://dx.doi.org/10.24382/4556
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A comparison of carbon sequestration potential and photosynthetic efficiency in evergreen and deciduous oaks growing in contrasting environments in the Southwest UK.

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A thesis submitted to the University of Plymouth in partial fulfillment of the requirements for the degree of:

D.Phil.

December 31st 2012.

Abstract

A comparison of carbon sequestration potential and photosynthetic efficiency in evergreen and deciduous oaks growing in contrasting environments in the Southwest UK.

Demelza Carne.

Global climate change is predicted to alter the weather patterns around the world, as climatic zones shift, forest carbon sequestration projects (e.g. the UK woodland carbon code) need to take into account the specific requirements of planted species. In the UK, oaks are an important charismatic group of trees favoured in recent planting programmes. The English oak (*Quercus robur* L.), has poor water conservation, but is a major component of natural forests in lowland UK. On the other hand, Holm oak (*Quercus ilex* L.) is a Mediterranean oak that has high water conservation and can also tolerate cold despite being restricted by minimum temperatures. At local scales, there may be advantages of planting either evergreen or deciduous oak species for forestry and climate mitigation. Alternatively, a comparative assessment of non native versus native productivity, may give clues to the invasiveness potential of Holm oak and its ability to out compete the deciduous oak along an urban to upland gradient. This thesis documents a series of field based experiments intended to analyse differences in carbon sequestration potential and photosynthetic efficiency between these two species and in relation to their environment within the Southwest UK.

520 one year saplings were planted, half in pots and half in nursery field beds situated on Dartmoor, the east Devon Dartmoor fringe, Totnes, and Plymouth city centre. Originally two sites were chosen for their relative 'urban' qualities, two at 'rural' localities, one upland and a control site with access to a polytunnel for comparisons with well-watered and non nutrient limited trees. However, data analyses showed that sapling characteristics were site specific with the five sites falling along an urban, rural to upland gradient. The field experiments included monthly height and diameters (ground level diameter or DAG), monthly assimilation rates and analysis of chlorophyll fluorescence to aid interpretation of photosystem II functioning and sapling 'vitality'. Further laboratory experiments analysed specific leaf area (*SLA*), mass based leaf Nitrogen (N_{leaf}) and carbon (C_{leaf}), with differences between sun and shade leaves included, to aid comparisons between species and sites. The final experiment was a destructive harvest and this was used to find total biomass estimates and carbon allocation to different root shoot fractions.

In order to quantify differences between saplings and adult trees a smaller experiment was conducted in the canopy using experienced climbers and leaf level productivity analysed. Intrinsic water use ($iWUE$), stomatal conductance (Gs), means net assimilation rates (A_n) and chlorophyll fluorescence parameters; Variable fluorescence over maximum fluorescence (F_v/F_m) and performance index (PI) were measured and relative carbon assimilation rates and productivity assessed and compared between species at one urban , rural and upland site.

Results showed that *Q. ilex* allocated relatively more carbon to branches and leaves as a sapling

which in turn increased growth rate and whole tree assimilation rates to larger values than the deciduous oak despite *Q. robur* being able to increase maximum assimilation rates in response to increasing temperatures. This gives *Q. ilex* the advantage and overall biomass was higher at all sites than *Q. robur* apart from the upland site where there were no differences in biomass accumulation between species. However, despite no significant difference in biomass at this site *Q. robur* had greater survival and photosystem II functioning. In mature trees *Q. ilex* was under stress and N_{leaf} and carbon sequestration potential were higher in the deciduous species at the urban site. In contrast, *Q. robur* was under stress at the upland site at Castle Drogo where thin and nutrient poor soils have made it more vulnerable to drought stress. Here, mature *Q. ilex* showed reduced photosynthetic efficiency in relation to cold and drought, but was able to recover when milder temperatures occurred. The results were site specific, with a reduction in both *SLA* and relative allocation to the leaf weight fraction (LWF) in *Q.robur* the only common urban related effect seen.

The potential for *Q. ilex* to perform well at sapling stage is due to its morphological plasticity and drought tolerance. This species may become more prevalent within the Southwest as local climates continue to push it northwards from its natural Mediterranean range. In contrast, if *Q. robur* continues to suffer from defoliation and fungal attack and this may leave it more vulnerable to competition throughout less fertile and drier areas of its natural range.

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Acknowledgements

I started this for Dad but finished it for you Mum, LOVE YOU LOADS.

I would like to thank my supervisors for all their advice over the past four years, in particular my Director of Studies, Dr Maria Donkin for her quiet patience and advice on writing up this thesis. Thanks also to Dr Steve Burchett and the Canopy research volunteers; Chris Harris, Valerie Maldeney, Sven Bakte, Matt Perry, Sophie, Rebecca and Adam Day, but especially Kiki O'Malley who I couldn't have completed the canopy work without.

Thank you to the undergraduate volunteers who helped with field work and the tedious biomass experiment, in particular Samatha Jones and Catherine Horrell. A very special thank you to Sarah Peake, who not only helped with field work but also carried out the second CN analyses for me when I was up to my ears in trees! Big thanks also to Mum, Debbie, Sharon, Norma, John, Nick, Sally, Geraldine and Rupert who helped pick leaves during the biomass experiment, I definitely couldn't have completed counting all those thousands of leaves without you.

Thanks to Moor Trees, a Devon based woodland charity and partner with Southwest OPAL (url: www.moortrees.org) for use of their tree nursery sites and to Dave Sharp at 'Digginit', a community organic allotment scheme, in Plymouth (url: www.digginit.org.uk). Paul Freeman, farm manager and volunteers at Dartmoor Prison Farm and Erica Rochester (private allotment owner) for use of allotment space for this project. Tom Wood, National Trust Ranger at Castle Drogo; Nick Butcher, Mt Edgcumbe Ranger and Gareth Harrison from Plymouth City Council for permission to use the mature tree sites.

This project was funded by the OPen Air Laboratories network, a nationwide lottery funded organisation dedicated to bringing nature to the wider community (url: www.opalexplorenature.org) and I thank them for the opportunity to conduct this research.

Most of all I am indebted to my amazing family, Frodo, Sam and Beren, for their tireless love and support.

Authors declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

Scientific seminars and conferences attended: The British Ecological Society; Forests and Global Change Symposium, University of Cambridge 28-30th March 2011. See below for conferences attended at which posters and presentations were given.

Signed: _____

Date: _____

Publications :

Posters and conference presentations :

2011:

SEB Joint Meeting with Biochemical Society and British Ecological Society Stress responses - molecules, organisms and enviroments *Charles Darwin House, London.* Poster presentation: **Photosynthesis and Efficiency in Evergreen and Deciduous oaks growing in Southwest UK.**

2010:

Climate Change Conference. *University College, Falmouth.* Oral Presentation. **Carbon Sequestration Potential in Evergreen and Deciduous Oaks.**

2009

SEB Annual Main Meeting 2009 *Scottish Exhibition and Conference Centre (SECC), Glasgow* Poster presentation: **Carbon Sequestration Potential in Evergreen and Deciduous oaks.**

Word count for the main body of this thesis: 42,750

Chapter 1

Introduction.

1.1 Climate change.

The last 14 years have included the eleven warmest years since 1850 (Hegerl et al. 2007) with climate forcing dominated by long-lived greenhouse gases (GHG). The global increase in GHG emissions was around 70%, between 1970 and 2004 leading to an average increase in temperature of 0.74°C between 1906 and 2005 (Hegerl et al. 2007). Carbon dioxide CO_2 is the most important anthropogenic GHG; pre-industrial levels of atmospheric CO_2 have risen from 280 ppm to the 2005 average of 379 ppm (Solomon et al. 2007). In 2004 CO_2 was responsible for 77%, of all GHG emissions in 2004 (IPCC 2007) and data from the Earth system Laboratory shows recorded globally averaged monthly means for CO_2 of approximately 395 ppm in January 2013 (over marine surface sites) (Dlugokencky and Tans 2013).
More importantly, the global rate of growth of CO_2 emissions has increased from 0.92 gigatonnes atmospheric Carbon per year (GtC) between 1995-2004 compared to the previously recorded rate of 0.43 GtC CO_2 per year between 1970 and 1994 (IPCC 2007).

As a result of excessive GHG in the earth's atmosphere the next 20 years could see an increase in temperature of 0.2°C per decade accompanied by changes in precipitation patterns around the world (Hegerl et al. 2007).

In the northern hemisphere, northern Europe is likely to experience an increase in winter rainfall and increased flash floods while southern Europe will be subjected to increasingly high temperatures and drought (Hegerl et al. 2007). In fact these changes are already documented such as increased drought in the Mediterranean (Solomon et al. 2009).

1.1.1 Climate change in the UK

All areas of the UK are predicted to experience reduced summer precipitation, an increase in summer and winter mean temperatures and an increase in winter precipitation under medium and high emission scenarios (UKCP 2009). In fact observed changes already show that between 1961 and 2006 winter rainfall increased in all regions while summer rainfall decreased (UKCP 2009). Minimum temperatures have increased for all regions of the UK and daily mean temperature has increased between 1.05 °C (Scotland) and 1.6 °C in London and the Southeast (UKCP 2009). Daily winter mean temperatures have shown the greatest increase of 2 °C in London and the Southeast, while Southwest UK has experienced an increase in winter mean temperatures of 1.7 °C, and annual mean daily temperature increase of 1.4 °C (UKCP 2009).

The Southwest UK is predicted to experience further increases in winter mean temperatures of 1.3 °C under the medium emissions scenario and increases in summer temperature of 1.6 °C, although annual mean precipitation is not predicted to change under this scenario there is likely to be an increase in winter precipitation of 7% and a decrease in summer preciptiatin of 8% under high emission scenario (UKCP 2009). However by 2080s these percentage increases will become more significant; precipitation levels increasing three-fold during winter and decreasing by a similar percentage during summer (24%) (UKCP 2009).

1.1.2 Climate change and forestry.

Leading on from the 1992 Earth Summit, the subsequent 'Kyoto Protocol'[1] 1998, imposed emission targets for industrialised countries to reduce their outputs of several important *GHG*; CO_2, Nitrous oxide N_2O, methane CH_4, and the fluorinated gases; hydro fluorocarbons (HFCs), perfluorocarbons (PFCs) and sulphur hexafluoride (SF 6) by 2008-2012; of these CO_2 is considered the most important (Alcamo et al. 1998).

Article 3.3 of the protocol allows industrialised countries (known as 'Annex B' countries such as the UK) to use afforestation, reforestation and land management strategies as mitigation tools (IPCC 2000; Brown 2002). Although it is also stressed that such tools should only be used in

[1] the Kyoto protocol became legally binding in 2005

conjunction with emission reducing tactics (van Minnen et al. 2006, 2008).

The seasonal flux of CO_2 as forests absorb CO_2 during the summer and release it over winter months, indicates the light and temperature response of vegetation in the absorption of atmospheric carbon (C) (Lorenz and Lal 2010). Deforestation emits 17.4% GHG annually and is the third largest emitter of GHG globally after energy use and industry (Betts 2000), having released 1.6 GtC annually during the last decade (Broadmeadow and Matthews 2003).

Despite such loses from deforestation and respiration, globally forests are a net sink (Jarvis and Linder 2007) and are estimated to remove around 2.1-3 GtC annually (Broadmeadow and Matthews 2003) nearly 10% of total terrestrial sink (Gough et al. 2008; Hegerl et al. 2007). The vast majority of carbon sequestered is stored as biomass in trees (90%) (Nowak and Crane 2002; Korner 2003). Watson et al. (2000) suggest that globally forests have the potential to sequester an extra 87 GtC by 2050, while UK forests are estimated to be sequestering carbon at a rate of 2.5 million tonnes of carbon annually (Cannell 2003) and utilise around 12% of UK landcover (Broadmeadow and Matthews 2003).

1.2 Climate and Tree Growth.

Tree growth is closely linked to climate, with tree distributions closely following climatic averages such as mean winter temperatures mean summer maximum temperatures and rainfall patterns. The effect of changing climates at regional level are already causing range shifts in broadleaved tree species such as beech (Jump et al. 2006). Evidence also suggests, evergreen broadleaved species are expanding their northern range as average minimum temperatures increase across central Europe (Berger et al. 2007). Historically tree species have expanded and contracted their range in response to changes in climate, in particular oaks have expanded from glacial refuges since the Quaternary era (Davis and Shaw 2001). Temperature and precipitation at the end of the previous growing season is important for tree growth, [e.g. for Beech (Kern and Popa 2007) , and oak (Drobyshev et al. 2008)] and changes in normal patterns at this time will exacerbate species ability to adapt to drought during the summer months.

1.2.1 Carbon Assimilation by Trees.

Trees assimilate carbon through photosynthesis and utilise 'dead' tissue to form part of their support structure (Franco 2008). The ability of trees to store carbon in such a way makes carbon sequestration by forests an important mitigation tool while other more permanent CO_2 sinks and new energies are developed (Korner 2003).

Carbon accounts for around 40% of a plants dry mass (Lambers et al. 2008) with The gross productivity of a forest ecosystem can be described as the sum of net primary productivity and autotrophic respiration as follows:- see equation 1.1 (in its simplest form).

$$GPP = NPP + Ra \tag{1.1}$$

Where GPP = Gross Primary production, NPP = Net Primary Production and Ra = autotrophic respiration (g$C\ m^{-2}\ yr^{-1}$) (DeLucia et al. 2007; Lindroth et al. 2008). However, disturbances that cause carbon release such as fire, harvest/management and pollution/transport effects should also be taken into account when measuring carbon balance in forests (Kennedy et al. 2008).

Photosynthesis

The photosynthetic apparatus of a tree is the leaf; during photosynthesis leaves convert light [Photosynthetically Active Radiation (P.A.R.)] into chemical energy and assimilate CO_2 from the atmosphere in order to synthesis carbohydrates (Scott 2008). The following equation gives the basic reaction of photosynthesis whereby CO_2 reacts with water and light energy (photons) to give carbohydrates and oxygen (O_2).

$$nCO_2 + 2nH_2O + \text{ light energy} \rightarrow (CH_2O)n + nO_2 + nH_2O \tag{1.2}$$

Where n is the number of molecules of each given element in order to produce the general structure of a carbohydrate. The most commonly used equation is that of glucose formation where $n = 6$.

CO_2 enters the leaf via tiny pores known as stomata. Stomata release water vapour and can control photosynthesis as they react to different environmental factors (Lorenz and Lal 2010). Leaf photosynthetic rate can therefore depend on many interacting variables such as the absorption of light (Nobel 2005), leaf age (Harper 1989), temperature (Filella et al. 1998), water availability and climatic extremes (e.g. Boisvenue and Running 2006; Waring et al. 2008), soil nutrients (e.g. Bergh et al. 1999) and the adaptability of species to their environment (Catovsky et al. 2002; Becker et al. 1994).

Water stress is a large factor in controlling CO_2 assimilation and changes in rainfall patterns at regional levels will affect carbon fluxes and assimilation (Franco 2008; Heimann and Reichstein 2008). Primary productivity is therefore limited by water availability (Amthor 1999). Increased temperatures will exacerbate water stress as transpiration rates are expected to increase (Heimann and Reichstein 2008). However, stomatal closure in response to increasing CO_2 and water stress mean that the overall effect may depend on soil water holding capacity and drought sensitivity of plants (Heimann and Reichstein 2008).

Respiration.

Respiration releases part of the CO_2 absorbed by the leaf in order to produce energy and carbon skeletons for growth (Cannell and Thornley 2000; Lambers et al. 2008; Gratani et al. 2008; Amthor 1995). Respiration is therefore an important component when calculating carbon sequestration and can be expressed as a ratio between NPP and GPP. The literature gives average respiration rates between 33 and 60% of carbon assimilated across a number of different environmental conditions (Clark et al. 2001; DeLucia et al. 2007; Litton et al. 2007; Gratani et al. 2008). Losses have even been estimated as high as 80% of total carbon assimilated (Amthor 2000). Respiration rates are affected by a number of factors including temperature, elevated CO_2 levels and severe water stress but can also change with species (Amthor 2000; Heimann and Reichstein 2008) giving an indication of the difficulties involved in measuring carbon sequestration rates in forest ecosystems (Luyssaert et al. 2007).

The ratio between NPP and Ra tends to increase with latitude while GPP remains relatively constant across latitudes (Valentini et al. 2000). Increased temperatures caused increased net

hourly CO_2 uptake in Boreal black spruce stand, however as temperatures rose above $20°C$ total CO_2 uptake declined (Grant et al. 2008). Despite GPP increases with rising temperatures optimum temperature for CO_2 assimilation in north temperate forests can be as low as $10°C$ (Luyssaert et al. 2007).

1.2.2 Species Choice and Productivity.

When deciding on which species to plant for carbon sequestration, the productivity of a species and its suitability to the environment in which it will be planted is important. At smaller scales carbon fluxes are more variable and total carbon pools depend on carbon allocation to roots, leaves and stems. Allocation and partitioning of carbon also depends on the availability of water, distribution and availability of resources, temperature and management regimes (Luyssaert et al. 2007) and can also depend on pollution stress. It has been predicted that trees in Northern Europe will become more productive with climate change, with an increased allocation of carbon to foliage (Alcamo et al. 2007). Monitoring sequestration potential at stand level using biomass estimates from height and diameter allometry are key methods in forest management decision and important tools for monitoring changes in productivity (Boisvenue and Running 2006).

In the Mediterranean, typical tree species are likely to change due to increasing temperatures and drought whereas in the UK some native species such as *Q. robur* (English Oak) may become less productive in southerly and lowland areas (Boisvenue and Running 2006). Willoughby et al. (2007) assessed the suitability of a number of species for planting farm woodlands at several different lowland sites in the UK, and concluded that species choice is important because of changing climate. Further, limited land availability brings with it a need to utilise a range of both urban and rural environments (Willoughby et al. 2007). As major lowland sites become hotter and drier and rainfall patterns change, the potential of non-native species to become more productive and therefore more efficient at sequestering carbon in these areas is a key area for research. However, the dangers involved in planting exotic species should also be taken into account with many non natives considered invasive (Rackham 2008).

1.2.3 Evergreen and Deciduous leaf habit.

The dominance of deciduous forests during a warm polar past was thought to be an adaptive strategy against high respiration rates during the warm polar winter (Osborne and Beerling 2003). However, Royer et al. (2003) found that warmer winters along with high atmospheric CO_2 in a simulated ancient polar environment did not increase respiration costs in deciduous species. However, compared with evergreen respiration costs for the same environment carbon lost through leaf litter gave evergreens the advantage thus reducing overall carbon sequestration in deciduous species by nearly half that of the evergreens in the ancient polar winter (Royer et al. 2003). However, despite the winter carbon costs discussed, and that deciduous species have less time in which to assimilate carbon over the growing season, they experience boosted photosynthetic rates towards the end of the summer and beginning of autumn (August - mid October) this leads to increased annual carbon uptake to similar levels as that of evergreen trees, despite leaf losses (Royer et al. 2005). More recently, research has shown that the deciduous species *Q. rubra* experienced greater assimilation of CO_2 to biomass but also had double the photosynthetic capacity than that of the evergreen hemlock in the same woodland (Catovsky and Bazzaz 2000; Catovsky et al. 2002).

The amount of carbon allocated to biomass in forests is around 50% of total biomass (Bateman and Lovett 2000; Litton et al. 2007). Recent information shows that UK forest ecosystems are storing an estimated 790 million tonnes of atmospheric carbon (MtC) with UK forests removing approximately 15 MtC annually in 2007 (Read et al. 2009). Over a period of five years (1997-2001) in the UK, an Oak woodland removed approximately 15 tonnes of CO_2 per hectare per year ($tC\ ha^{-1}\ yr^{-1}$), compared with 24 $tC\ ha^{-1} yr^{-1}$ in a Sitka Spruce stand (Read et al. 2009) although the lifetime of an oak tree is longer than Sitka spruce and brings other benefits such as increased biodiversity. Recent past UK plantation forestry has relied on non native conifers such as Sitka spruce, further, woodland planting schemes have been concentrated on upland sites and other environmentally sensitive areas, although planting is now restricted to below 240 m above sea level (masl) (Cannell and Dewar 1995). Further to this as the age structure of UK woodlands change, carbon sequestration in these woodlands is likely to decline as low

as 4.6 $MtCO_2\ yr^{-1}$ by 2020 (Read et al. 2009) despite the Forestry Commission grant schemes for planting native broad-leaves and restoring native woodlands available since 1995 (Cannell and Dewar 1995). Therefore it is important that the carbon sequestration potential of individual species is measured and in relation to a variety of environmental conditions. Carbon sequestration projects should also be aware of the effectiveness of both prudent site and species choice not only in order to provide accurate information for evaluating the UK carbon pools, but also in order to promote the effective use of the license given by the United Nations Land Use, Land Use Change and Forestry (LULUCF) advice for afforestation and reforestation practices (IPCC 2000).

Carbon Nitrogen Ratios, Specific Leaf Area SLA.

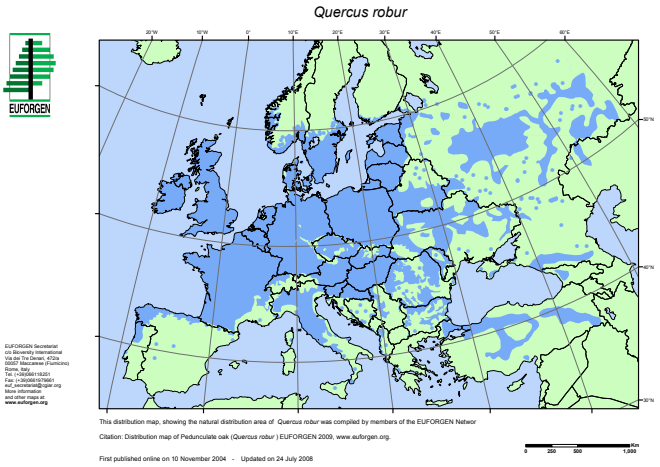
The carbon allocated to the living part of a tree remains relatively constant after canopy closure (Franco and Kelly 1998; Franco 2008). Therefore, leaf area and mass are important components in measuring productivity, and specific leaf area (SLA) is a good representation of biomass/carbon sequestration (e.g. Lindroth et al. 2008) although climate induces variation in SLA (McCarthy et al. 2006). Larger trees are able to assimilate more CO_2 due to the relatively large leaf area compared to that of smaller trees (Gratini and Varone 2006) but photosynthetic efficiency of leaves is also important. In order to measure photosynthetic efficiency and carbon sequestration potential in species all these factors therefore need to be taken into account. Species with evergreen or long term leaf habits require greater carbon allocation to cell structure, resulting in a higher mass per unit leaf area (LMA) and lower leaf nitrogen per unit area (N_{leaf}), in turn leading to lower specific leaf area and lower gas exchange rates per unit area (Reich et al. 1999). Therefore photosynthetic nitrogen use efficiency (PNUE) is lower for evergreen species; Takashima et al. (2004), studying evergreen and deciduous oak trees, found a high correlation between photosynthetic capacity (A_{max}) and leaf nitrogen concentration (N), finding that evergreen broadleaf oaks tended to have low specific leaf area (SLA) and lower photosynthetic capacity per unit leaf *N* than deciduous oaks due to increased allocation of photosynthetic *N* to cell walls as a mechanical aid to leaf longevity (Takashima et al. 2004). In contrast, deciduous leaf habits tend to allocate greater *N* to photosynthetic apparatus and a higher SLA in turn

leading to greater gas exchange rates and lower root costs during winter (Takashima et al. 2004). However, this advantage is lessened by the susceptibility of deciduous leaves to herbivory and the environment (Reich et al. 1999). A review by Givnish (2002) showed longer photosynthetic season, lower construction costs, lower costs for nutrient replacement, and tougher laminae (thus better forest resistance against drought and herbivory) of evergreen leaves to be an advantage. In contrast, previous authors (e.g. Miller and Stoner 1979; Reich et al. 1999) have shown increased construction costs in evergreens in the field. Villar et al. (2006) found that construction costs were not significantly different in contrasting leaf habits but these costs differed with phylogeny, suggesting tradeoff between defense mechanisms and growth. In perennial species, typical of seasonal environments, current leaf growth is assimilated from the previous year's photosynthate (Harper 1989). Trees belonging to the genus *Quercus* spp. are 'ring porous'; where a ring of large pores develops early during annual growth; this porous ring is then followed by a ring of smaller pores later in the annual cycle (Morecroft et al. 2003). As a result new wood is formed around 2-3 weeks before budburst which in turn reduces the available resources for leaf development (Morecroft et al. 2003). Spring flush in European oaks also relies on the mobilisation of stored reserves from the previous year's assimilation (Both and Bruggemann 2009).

1.2.4 Evergreen Vs Deciduous Oak for Carbon Sequestration in UK.

The distribution of *Quercus spp.* is linked to water availability and soil fertility (Breda et al. 1993). In the UK there are two native oaks, *Q. petrea* (Matt) Liebl. (Sessile oak) and *Q. robur* (Pedunculate oak). Pedunculate oak preferring more fertile soils (Breda et al. 1993; Becker et al. 1994) and more tolerant of water logged soils than the Sessile oak (Morecroft et al. 2009). The Mediterranean evergreen, *Q. ilex* (Holm oak) is widely planted in urban and parkland areas and has become naturalised along the South coast of England (Peterken 2001). *Q. ilex* prefers sandy limestone soils, tolerating more clayey soils when average rainfall is more than 300mm (Barbero et al. 1992). Although in its natural range, *Q. ilex* sub species *ilex* is found in more coastal locations while the sub species *rotundfolia* is found inland, it has been suggested that *Q. ilex* represents one complex species and grows in semiarid, sub-humid, humid and per-humid

(a) Natural distribution map for *Q. ilex* (Figure 1.a has been removed due to Copyright restrictions)



(b) Natural distribution map for *Q. robur*

Figure 1.1: Natural distribution maps for a) *Q. ilex* ((Removed due to Copyright restrictions please see text for description of *Q. ilex* natural range, original figure from (Welk et al. 2013)) and b) *Q. robur* (blue area shows distribution, map from (EUFORGEN 2009).

climates but prefers the sub-humid climate where it can occur in a wide range of temperatures and soils (Barbero et al. 1992). In colder climates however competition from deciduous oaks and conifer species mean *Q. ilex* restricted to areas where competition is less such as rocky outcrops and poorer soils (Barbero et al. 1992) (see Figure 1.1).

Q. robur is native to the UK although its natural range extends throughout Europe, SW Asia and North Africa (Kleinschmidt 1993). Although *Q. robur* is thought to have suffered unfavourably in the changing climate of the last 150 years compared to *Q. petrea* (Epron and Dreyer 1993*b*; Becker et al. 1994; Beckett et al. 2000) and is considered drought sensitive (Dickson and Tomlinson 1996). However, there is evidence that *Q. robur* is becoming more productive in the upland regions of the UK due to changing regional climate (Chavana-Bryant 2006 unpublished masters thesis).

Q. ilex subsp. *ilex* is a Mediterranean schlerophyllus species and in its Mediterranean range tends to be restricted by water availability and winter temperatures, it has been shown that *Q. ilex* has a higher tolerance to winter freezing than to summer drought (Ogaya and Penuelas 2003). However, in contrast, others have shown that *Q. ilex* was limited more by winter freezing than by summer drought (Nardini et al. 2000). More recently Gimeno et al. (2009) showed how populations of *Q. ilex* were able to tolerate climatic extremes throughout their Iberian range, without intrinsic adaptation. Using leaf area (LAI) as a surrogate for shoot biomass Crescente et al. (2002) found a 20% reduction in photosynthetic efficiency and lower relative growth rates for *Q. ilex* during low temperatures in part of its native range in the region of Lake Garda, Italy. Net photosynthetic capacity and stomatal conductance was also reduced in *Q. ilex* subjected to severe drought, closing stomata as a defence mechanism for water stress (Filella et al. 1998). In terms of range shifts, it has been widely discussed that *Q. ilex* is becoming restricted in driers areas of its native range and that wetter sites are likely to become more productive for this species in the future (e.g. Sabaté et al. 2002).

1.3 Species and Environment.

1.3.1 Urban Forestry.

The urban environment presents challenges to plant growth. Air pollution combines with poor soils, and limitations in water availability due to restricted rooting depths, impermeable surfaces and building cover.

Plant responses to air pollution involve stomatal regulation and increases in drought sensitivity (Chappelka and Freer-Smith 1995; Calatayud et al. 2011). Increased ozone exposure can lead to stomatal dysfunction and subsequent drought sensitivity (Chappelka and Freer-Smith 1995). Increased stomatal regulation and blockage can lead to reduced photosynthesis with leaf age and reduced transpiration rates and eventually premature leaf senescence (De Nicola et al. 2011). In contrast, nitrogen dioxide (NO_2) pollution has been shown to be utilised by plants and broken down into products used in amino acid formation (Nowak and Crane 2002), thus increasing carbon assimilation in some instances.

Urban environments tend to have higher CO_2 concentrations and higher mean temperatures accompanied by a smaller diurnal temperature range (Searle et al. 2012). Trees then have the potential to sequester more carbon and can also have a cooling effect on urban environments by providing shade, while transpiration can cool air under canopies (Gratani and Varone 2006). Both deciduous and evergreen oak contributed to decreasing air temperatures in Rome through shade and transpiration effects, however, deciduous oaks had a greater effect than *Q. ilex* probably due to the larger crown area (Gratani and Varone 2006). However the same study found that *Q. ilex* had greater overall *C* sequestration due to its longer photosynthetic season.

Planting trees in urban environments is also beneficial to health and social well-being of urban dwelling humans thus having indirect affects on a country's carbon use (e.g., see Westphal 2003).

1.4 Aims and Objectives:

The overall aim of this research was to test the hypothesis; The native hardwood *Quercus robur* L. has greater carbon sequestration potential and photosynthetic efficiency than the Mediter-

ranean species *Q. ilex* L. , in different rural, urban and upland environments in the Southwest UK. Experiments were conducted on one to three year saplings : Differences in height and diameter growth, relative growth rate, biomass, leaf level carbon assimilation and photosynthetic efficiency and leaf traits were assessed. In order to aid scaling to older trees, a smaller experiment was conducted on mature trees: photosynthetic rates, photosynthetic efficiency and leaf traits were measured in the canopies of mature trees at rural, urban and an upland location.

1. General working hypotheses

    1.1. There is a significant difference between *Q. ilex* and *Q. robur* in productivity and carbon sequestration potential in Southwest UK.

    1.2. Overall productivity, total biomass and carbon content is greater in *Q. robur* than *Q. ilex* and there are differences in these parameters between the urban, rural and upland environments.

    1.3. There are differences in assimilation and photosynthetic efficiency in young saplings compared to mature trees.

Further analyses on the differences between sun and shade leaves of each species and their position in the canopy were incorporated into the final discussion on productivity/carbon sequestration potential of each species in relation to their environment.

1.5 Overview of Chapters

Chapter 1 Chapter one is the introduction and gives a brief overview of anthropogenic climate change and tree growth. It then moves on to discuss the relative benefits of evergreen and deciduous leaf habits in broadleaved trees in productivity and carbon assimilation. There is also a short section discussing planting in urban environments.

Chapter 2 Chapter two is the general methods section. Where the seedling sites are introduced. Growth, photosynthesis, chlorophyll fluorescence and light response curves, biomass and environmental methodologies are also introduced.

Chapter 3 This chapter covers the measurements of the environmental parameters. Soil nutrients, moisture and pH; Nitrogen dioxide diffusion tube analyses; Climate statistics shown as monthly means for each year, 2009 - 2011.

Chapter 4 Chapter four covers direct measurements of photosynthetic rate. Experiment one gives light response curves for each species followed by an indication of both light saturated diurnal rhythms (using a dimorphic light and a steady photosynthetically active radiation (PAR) of 500-600 $\mu mols\ m^{-2}\ s^{-1}$) and diurnal rhythms using natural light.

Night time respiration rates have been recorded and used to complete light response curves and as an aid to interpretation of carbon assimilation in chapter 3.

There then follows a drought experiment which gives species response to experimental drought and this is again used to interpret effects of each site environment on photosynthetic parameters.

Results from the ongoing recording of sapling photosynthesis and chlorophyll fluorescence give monthly differences between species and sites and data from the control site gives annual fluctuations of each species in well watered nutrient rich environment.

Chapter 5 Chapter five analyses leaf traits in saplings of both species. N_{leaf}, C_{leaf} and *SLA*. N_{leaf} is closely correlated with maximum assimilation rates and can also be altered by environment through changes in light. In some cases urban pollution can cause *N* deposition to leaf and *N* accumulates, this can be used in biochemical reactions and subsequently reused in carboxylation process (see chapter for relevant references). *SLA* also varies with environment as does (although to a lesser extent) C_{leaf}. These traits can then be used to aid the prediction of productivity in relation to environment for each species and this is discussed.

Chapter 6 Chapter six is concerned with growth rates of the saplings and relative growth rates using height and diameter data

Specific leaf area and carbon nitrogen ratios are presented and finally results from the biomass study where 200 three year old saplings were destroyed and split into root, stem, branch and foliage fractions, dried and weighed for biomass.

Allometric equations were determined using total biomass (TB), above ground biomass (AGB), height (H) and diameter at ground level (DAG) data and differences between species and sites discussed.

Chapter 7 Chapter seven presents the results of the mature tree experiment includes measurement of photosynthetic rates, chlorophyll fluorescence parameters, Specific leaf area and carbon nitrogen relationships. Ontogenetic differences are left for discussion in the final chapter.

Chapter 8 Chapter eight gives the final discussion on differences in carbon assimilation and sequestration potential of the two species and compares sapling and mature tree data and discusses issues of scaling for saplings to mature trees.

Chapter 2

General Methodology.

2.1 Local Climate.

The general climate of Southwest England is described as oceanic (warm temperate, fully humid with warm summers and cool winters) (Kottek et al. 2006). Rainfall is variable throughout the region. Mean annual rainfall (1961-1990) was around 800-1000mm per annum for lowland Plymouth area (urban sites) increasing to between 1000-1200 for South Brent/Totnes area (two rural sites) and 1800-2000 for the High Moor at Princetown (third upland site) (Phillips and Denning 2007; Phillips and Mcgregor 2002). The changing climate affects precipitation and temperatures and more recent seasonal rainfall trends show Plymouth has become 50 mm a year wetter during winter and 30 mm drier during summer over the past 150 years along with a significant increase in winter average air temperatures ($p < 0.05$) for Plymouth (following national trends) (Wood 2004). Soils of all sites, except for the plots at Dartington (DT) and Dartmoor Prison Farm (DPF), are similarly described using the Cranfield University 'Soilscape' online tool (National Soil Resources Institute 2009); free draining, slightly acidic, base rich and with low fertility. For DT, soils are described as free draining, slightly acidic but with high fertility. Soils around the plot at DPF are described as free draining, acid and loamy soils over rock with areas of slowly permeable, wet and very acid upland soils with a peaty surface. However each site and specific plot differs in past soil management and further soil characterisation has been completed for each site (see results section for detailed, site specific assessment of soil characteristics, rainfall, and air temperatures).

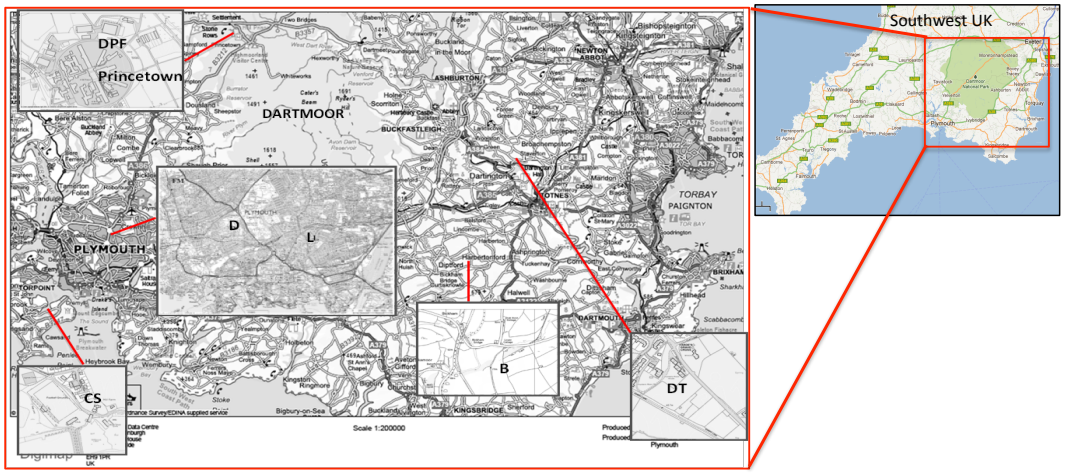


Figure 2.1: Location of Study Sites: Urban sites, Saltash Rd; Laira (L) private allotment on Norfolk Rd, Laira; Digginit (D) community garden, Stoke. Rural sites, Broadley (B) 'Moortrees' allotment, near South Brent; Dartington (D), 'Moortrees' allotment near Totnes. Upland site, Dartmoor Prison Farm (DPF), Princetown. Control site, Millbrook SE Cornwall where saplings were kept in Polytunnel (Digimap 2009)

2.2 Site Descriptions.

2.2.1 Upland Site.

Dartmoor Prison Farm (DPF).

HMP Dartmoor Farm, Princetown, Yelverton, Devon (SX585740) is situated on the high moor at an altitude of approximately 400m.a.s.l. Soils are shallow upland soils and the study plot itself has a peaty top soil added. Overall acidity is pH 5 and the topsoil contains high levels of nitrogen but with relatively low mineral nitrogen.

2.2.2 Rural sites.

Broadley (B).

Moor-Trees, Broadley Nursery (B) is situated in a valley, approximately 80 m.a.s.l near Totnes on the moorland edge (SX 727 553). The nursery is the fenced corner of a field containing semi-permanent grassland. The study plot at B is situated near the bottom of a shallow north-west facing slope.

Dartington (DT).

Moor Trees Hunters Moon Nursery (DT), Dartington, Totnes (SX 791 614) is approximately 30 m.a.s.l, situated above the A485, on a gentle north-north-east facing slope. The land is old agricultural land belonging to the Sharpham Estate, and once contained chickens. Past land use affects the fertility of this site and there are many 'bones' found throughout the upper soil layer.

2.2.3 Urban sites.

Digginit (D).

The community allotment at Digginit (D), Raynham road, Plymouth (SX467555) is approximately 40 m.a.s.l and is situated behind (<0.75 km) two of Plymouth Cities busiest roads (B3396 and A386). The tree saplings are planted near the top of an east-facing slope with a low mineral N content, pH 6.4 and TOC of around 5.3%.

Laira (L).

Norfolk Close council allotments near Old Laira Rd (L) (SX 502 559) is approximately 70 m.a.s.l and are situated at the top of a steep east south east facing slope approximately 60 m.a.s.l. Soils have an average soil pH of 6, a low TOC and nitrogen content.

Control Site (CS).

The control site (CS) is situated in Millbrook, SE Cornwall (SX428525). CS is a lowland rural site less than 10 m.a.s.l, flat, sheltered but with exposure to sunlight during the day. The control site also has access to a poly-tunnel during Autumn, Winter and Spring months to reduce affect of minimum temperatures (although tree saplings were taken out of poly-tunnel during peak summer 2009 due to high maximum temperatures they remained inside the polytunnel for subsequent summers due to rooting. Tree saplings at CS are potted (P); there are no planted (G) saplings and this site was mainly used as a reference site for saplings that had been watered and kept in favourable conditions, the control site was also used for respiration, light response curves and diurnal rhythm experiments.

Figure 2.2: Sampling design showing saplings grown in blocks of twenty saplings of each species in pots (P) and field grown (G) (site shown is rural DT)

2.3 Sampling protocol.

One year old saplings of the UK *Quercus robur* L. (Pedunculate oak) were obtained from the tree planting charity 'MoorTrees' [1] as bare root stock (provenance: Hembury wood, classified as western Atlantic oakwood and situated on the SE Dartmoor fringe). The Mediterranean *Q. ilex* sub-species *ilex* (Holm oak) was purchased in one litre vermiculite/peat based compost as one year out door hardened seedlings (provenance unknown: located from SE UK, Sussex) (nomenclature for both species follows (Stace 1997)). Saplings were planted over the winter, between December 2008 and January 2009, when growth was expected to be at a minimum.

Forty saplings of each species were planted at each site in a three-factor block design, where SPECIES, SITE and SOIL CONDITION were factors and soil condition had two levels: field grown (G) and potted (P).

Each soil condition (treatment) contained 20 saplings. Twenty saplings of each species positioned side by side in 10 litre (L) pots and 20 of each species planted in the ground at 30-40cm spacing (the same distance as the 10L pots standing side by side). This was the same for all sites apart from the control site CS.

The Control site (CS) had 20 of each species in 10L pots and no planted saplings due to both space within the poly-tunnel and economic constraints.

The pots contained a similar compost/soil mix with an even distribution of slow release fertilizer (Osmocote Exact Low Start 16-18 month). Soils at all planted sites were characterised according to Cranfield University's 'Soil-Scape' and laboratory analysed for moisture content, pH and nutritional status.

Due to issues with rooting and subsequent comparisons with field grown saplings, the potted saplings were not periodically re-arranged to alleviate competitive differences in growth between interior and exterior saplings.

[1] Moortrees is based in South Brent, Devon (URL: http://www.moortrees.org/

2.3.1 Sampling Protocol for Soils.

Soils at each site were sampled using a systematic sampling technique (as suggested for level II soil analysis for monitoring the effects of air pollution on forests (United Nations 2006). A soil auger was used to remove 0-10cm depth of soil from 10 places within the 2x3m plots; the soil was then mixed thoroughly, bagged and labeled for analysis. This procedure was then repeated at another depth of 10-20cm in order to give an idea of the changes in available soil nutrients through the soil profile.

Soils were then prepared for laboratory use by splitting each mixed site sample into two. Half of the soil for each site was air-dried, sieved and ground in a 2mm sieve. The other half of the soils were kept moist for analysis of water content, soil pH. and determination of total nitrogen (TN) by the Keijdhal method (Tan 2005).

Soils were tested for total nitrogen (TN), soluble inorganic Nitrates (mineral Nitrogen) analysed as (N); available potassium (AK) using exchangeable cations in soil; available phosphate (AP) (using Brays No. 1 extractant), pH using a pH meter and di-ionised water, total organic carbon (TOC) using gravimetric method, and $\%H_2O$ in wet soil , $\%H_2O$ in dry soil .

2.3.2 Laboratory Methods for Soils.

Soil Moisture Content.

To give a direct indication of water content of the soils at each site, the wet mass percentage of water lost was determined using the 'gravimetric' method.

Soil moisture content was found by weighing xg of fresh, moist soil in a weighed foil wrap and then drying in an oven at 70°C for 24 hrs repeatedly until two consecutive dry weights were the same [methodology follows Tan (2005)]. The following equations show how a percentage for soil moisture content was found. Eqn. 2.1 gives the amount of actual water H_2O lost during the drying process while Eqns. 2.2 and 2.3 give an indication of the H_2O content of the soil expressed as either percentage of dry mass or wet mass. For the purpose of this experiment only wet mass % H_2O was determined using eqn. 2.3.

$$H_2O\ lost = weight\ of\ moist\ soil(g) - weight\ of\ oven\ dry\ soil(g) \tag{2.1}$$

$$Dry\ mass\%H_2O = oven\ dry\ mass - wet\ mass/mass\ oven\ dry\ soil(g) \times 100\% \tag{2.2}$$

$$Wet\ mass\%H_2O = (wet\ mass - oven\ dry\ mass)/mass\ oven\ dry\ soil(g) \times 100\% \tag{2.3}$$

(Tan 2005).

Soil Acidity/Alkalinity (pH).

pH. is determined by the Log_{10} hydrogen ion H^+ concentration in soil solution and was determined on soils direct from the field using a pH meter and de-ionized water as a reagent. pH determines nutrient availability in the soil, therefore pH was measured before determining the method of extraction for N, P and K.

Total Organic Carbon (TOC).

Total Organic Carbon (TOC) was determined using the dry combustion method. TOC was measured as an indication of organic *C* present: none of the soils were alkaline or were formed from a calcareous base rock, therefore inorganic carbon was assumed to be insignificant. Approximately 2g of oven dry soils were weighed and placed into the Skalar Primacs SLC Analyser where CO_2 is measured, by 'Non Dispersive Infra Red Detection (NDIR) for Total Carbon, at a temperature of 1043°C for 6 minutes per sample. At this temperature the carbon is completely oxidized to CO_2 in the presence of a catalyst.

Nitrogen.

Nitrogen is found in soils in both organic and inorganic forms and is an essential nutrient for plant growth chlorophyll and protein formation (Tan 2005).

Plants take nitrogen from the inorganic source while organic nitrogen is present in soil as a nutritional reserve, released after decomposition and mineralisation of organic matter (Tan 2005).

Organic N is closely associated with soil organic matter with only 2-3% of organic N mineralised and released in inorganic form (Tan 2005).

Total N (TN).

TN was determined by the wet oxidation method (Kjeidhal) due to this methods applicability to samples low in N (see appendix A for full schedules on nitrogen extraction methods).

Mineral N (MN).

Mineral or Inorganic MN is composed of Ammonia $NH_3 - N$, ammonium $NH_4 - N$, Nitrates $NO_3 - N$ and Nitrites $NO_2 - N$, these types of inorganic N are continuously formed by decomposition and mineralisation of organic matter via the nitrogen cycle (Tan 2005). Most of them are soluble in water and their concentration in soil depends usually on the application of fertilizers (Tan 2005). MN was analysed using soils moist from the field and potassium chloride used to extract the soluble and exchangeable nitrate and ammonium ions (see Appendix A for detailed schedules).

Available Phosphorus (Phosphates).

Methodology for determining available phosphorus depends on the pH of soil. For the purpose of this research the Bray 1 method for pH <6 soils was used. The Bray 1 method is used for the determination of available P (phosphorous soluble in dilute Hydrochloric acid (HCl) and ammonium fluoride (NH_4F) and the extractant is designed to remove acid-soluble P and a portion of the phosphates of Al and Fe.

Available Potassium (AK) (Measuring Exchangeable Cations).

AK was analysed as exchangeable K^+ ions, using acetone as a reagent $[OC(CH_3)_2]$.

For full schedules detailing all soil analyses see appendix A.

2.3.3 Sampling Protocol for Growth, Biomass and Measurements.

Growth (Height and Diameter).

Height, measured from root collar to tip of central stem (H) and mean diameter at ground level (DAG) was measured for all saplings. Height was measured using a meter rule attached to a wooden disk with a piece cut away in order to reduce chances of error due to different volunteers sampling techniques. Diameter was taken using a metal dial caliper accurate to 0.02 mm and was taken 1cm from ground level (if saplings had root collar above ground level then DAG measurements were taken above the root collar) and again at right angle to the original measurement. Measurements were taken every four weeks between planting and harvest, August 2011.

Leaf Traits.

On one day in August 2009, one single mature, current year leaf was taken from the upper canopy from 10 trees at each factor and treatment level (no distinction between upper and lower canopy at this stage in growth).

This experiment was repeated at the end of August 2010 and again in June 2010. However this time either two or three leaves were taken from each of the 10 trees per treatment. One current year mature leaf (CYM) from the upper canopy and one CYM from the lower and if any immature (NL) leaves existed then one of these was also taken from the upper canopy.

During the initial leaf collection (2009), each leaf was drawn around using graph paper (2, 10 and 20 mm squares ruled). Length (L) from petiole to tip and width (W) at widest point were recorded and leaves divided into easily measurable shapes (see appendix A) then leaves placed on a leaf area machine, (Li-Cor) calibrated with a $25cm^2$ square accurate to $24.7cm^2$, and their true leaf area determined. Subsequent years, equations formed from initial leaf area estimates were used to find leaf area (LA).

Leaves were weighed, dried at 60°C, and reweighed. Differences in specific leaf area (SLA), leaf mass per area (LMA) were looked for between sun (upper) and shade (lower) leaves in two, three and four year old saplings. Mature current year leaves (CYL) from 2009 and 2010

were also analysed for carbon and nitrogen ratios using the Elemental Analyser EA1110 that measures carbon C, Nitrogen N and Hydrogen H percentages per 2mg of sample. Carbon and nitrogen were then analysed individually as percentages and as a C/N ratio.

Biomass and total leaf area.

On 22nd September 2009, eleven *Q. robur* and seven *Q.ilex* saplings taken from rural site B were subjected to destructive harvesting for biomass and total leaf area calculations. The saplings were cut into root, stem, branch and leaf fractions. Each fraction had initial weights taken, and were then oven dried at 60°C until two consecutive weights the same were recorded (after approximately one week).

Before weighing and oven-drying the leaf fraction, leaves were divided into mature CYL and immature NL and after initial weighing total leaf area calculated by a LiCor Leaf area machine (calibrated to 24.8cm^2).

After drying, oven dry weights were calculated for each of the fractions and total biomass (TB), above ground biomass (AGB), total leaf area (TLA) and SLA were calculated to enable calibration of allometric equations using biomass, leaf area, and height and diameter data.

Allometric Equation for use with growth parameters and biomass relationships.

The literature contains a huge amount of information on allometric equations for *Quercus* species, however many of these data are only valid for mature trees with diameters 7cm or greater (Ravindranath and Ostwald 2008).

An equation in the form D_2H has been used for sapling biomass studies in various forms (Williams and McClenahen 1984 e.g.), in particular is the use of ground level diameter (DAG) as a substitute for DBH in sapling based allometry. Williams and McClenahen (1984) found it to be useful in biomass estimates for White and Black Oaks from eastern America using common logarithms. Eqn. 2.4, using natural logs to homogenise variances, was used to determine allometric biomass equations for saplings in this study.

$$Log_e Y = b_0 + b_1 Log_e(D_2H) \tag{2.4}$$

(Williams and McClenahen 1984).

Final harvest began the last week in August 2011 where approximately 10 saplings from each site and condition were destroyed and split into the same fractions as the previous biomass experiment. New allometric equations were prepared using the same basic equation 2.4. Carbon content was also estimated from dry weights of each fraction and allometric equations derived for each species and site.

Difficulties in removing whole root systems without loss with the additional difficulty of cleaning soil stones from roots mean that often below biomass estimates are removed from final analyses. However in this instance, roots were dug out from field grown saplings to a depth of 50 cm and radius from stem base of 25cm in an attempt to reduce the error across samples. Some fine root fraction was lost during cleaning due to time involved in sieving through soil debris, however, the general aim was to retrieve 75+% of fine root fraction contained in soil waste. It is accepted that fine roots below 1mm in diameter are under sampled (Poorter et al. 2012) and total biomass TB was analysed with this error in mind.

For saplings that had over 600 leaves, total leaf area was determined in the final harvest using the individual leaf areas of the first 100-200 leaves from upper, middle and lower canopy of each tree and averages used to estimate total leaf area of each tree.

2.3.4 Sampling Protocol for Environmental Parameters.

In January 2009 rain gauges were set up at two rural sites; Broadley and Dartington and monthly averages worked out from the data collected. Rainfall data for Dartmoor prison farm and Plympton (as a reference rainfall for the South Devon sites) was received monthly (as daily averages) from Environment Agency (EA) and data between January 2009 and October 2009 were converted to monthly averages.

Rain data for Urban sites was taken from the University of Plymouth ‘Metnet’ weather station archive (Metnet 2009).

Temperature and relative humidity (RH) were also recorded at all sites using Thermochron ‘Ibuttons’ (Homechip Ltd). Thermochrons recorded hourly temperature and humidity data from

two ibuttons per site.

All the climate data collected between January 2009 and August 2011 was converted into monthly averages and converted into a climate graph (see Chapter 3 for yearly/monthly climate averages at each site).

2.3.5 Sampling Protocol for Pollution Parameters.

Nitrogen dioxide (NO_2) diffusion tubes (Gradko Environmental) were placed, one at each site, $1.5m$ from the ground as close to the research plot as possible (<10m). Tubes were sent back to Gradko Environmental Herts for analysis of monthly NO_2 levels (measured in μgNO_2). Overall average monthly NO_2 was worked out from monthly data collected between June 2009 and June 2010 (although some months were missing).

2.3.6 Sampling protocol for gas exchange and chlorophyll fluorescence.

Gas Exchange.

Gas exchange parameters were assessed throughout the sampling period using a LCi portable Infrared-Gas-Analyser (IRGA) (ADC BioScientific, Herts), accompanied with a 'broadleaved' leaf chamber.

Flow-rate was set at 200 $\mu mol\ s^{-1}$ and stabilisation of sub-stomatal CO_2 was used as a guide to settling time for analysis, this taking about 20-40 seconds.

Parameters used were; mean net photosynthetic rate (A_n) $\mu mol\ m^{-2}\ s^{-1}$, mean stomatal conductance of CO_2 (Gs) $mol\ m^{-2}\ s^{-1}$ and mean transpiration rate (E) $mmol\ m^{-2}\ s^{-1}$.

Monthly A_n.

The IRGA was used with a Light-Unit attached (ADC Scientific, Herts), this was used to alleviate the effects of variation in light levels during sampling. Unless used for light saturation experiments, the light unit was used at approximately $490-520$ $P.A.R.$ for consistency in data across sites.

To obtain monthly averages for each site A_n measurements were taken at each of the seven sites from 10 trees in each species/condition. Five mature leaves were chosen per tree on one day

each month (this was subject to weather therefore for some months data is missing). If there was a distinction between upper and lower canopy (which in some cases and mostly for *Q. robur* this was not obvious) then two upper and two lower mature leafs were measured.

A_n data was downloaded and used to ascertain differences in A_n across sites, species and conditions and over time using a general linear model (R 2009). The relationship of A_n to biomass was also considered.

Light Response Curves.

In order to assess the light saturation point for each species light saturation curves were produced in the middle of February and again in April 2011 for *Q. ilex* only, and for both species in August 2010. (see results section, chapter 4 for curves).

The ADC Light-unit was used to measure A_n at a number of different light levels using three neutral density (ND) filters (optical densities, 0.1, 0.2, and 0.3) (ADC Bioscientific, Herts). The ND filters were used in combination allowing 79.4% (0.1), 63.1% (0.2) and 50.1% (0.3), 39.8% (0.1 + 0.3), 31.6% (0.2 + 0.3), 25% (0.1 + 0.2+ 0.3) of light to be transmitted onto the leaf for each combination.

Each light saturation experiment was conducted at the control site CS.

Three leaves from two healthy *Q. robur* saplings and two healthy *Q. ilex* saplings were measured at eight different light levels using the ADC light unit and natural density filters (see appendix G for specific light levels and densities used). The LCi IRGA was configured for use with a light unit at $12.5v$ (Hfactor = 168, TRW = 0.9).

(See results section 'Light Saturation Curves').

Diurnal Rhythms.

Diurnal rhythms for *Q. ilex* and *Q. robur* were accumulated over two days during August 2009 and 2010 (see chapter 4).

Four leafs (2 x upper, 2 x lower unless no distinction made) from each of five *Q. robur* and five *Q. ilex* trees were measured between 5:00 am and 16:30 pm. Each set of measurements took

roughly 20 minutes and was repeated every 30 minutes throughout the day.

This experiment was repeated during June 2010 using the light-unit set at 500-550 PAR (see chapter 4 on photosynthesis).

Chlorophyll Fluorescence.

Chlorophyll fluorescence was measured using the Pocket PEA (Hansatech Instruments Ltd, Norfolk).

At each site, four leaves from each of ten trees, per species and treatment were sampled.

Leaves were first covered with leaf clips and dark-adapted for 30 minutes to ensure full dark adaption. Each leaf was then subjected to a bright flash of light at $3500 molss^{-1}m^{-2}$. The pocket PEA records a number of parameters over a period of a few seconds; F_0 (which is the value of fluorescence at time 0 just after full dark adaption), F_m which is the value of fluorescence at saturating light (maximum excitation), and F_v which is variable fluorescence and indicates maximum capacity for photochemical quenching, and is calculated by $F_m - F_0$ (Cavender-Bares and Bazzaz 2004).

Each of the measured parameters has meaning but the most commonly used chlorophyll fluorescence parameter used within plant research is the variable fluorescence maximum fluorescence ratio (F_v/F_m). The ratio F_v/F_m was then used to indicate the maximum quantum efficiency of Photosystem II. F_v/F_m is a sensitive indication of plant photosynthetic performance, with 0.85 being the maximum obtained in a healthy plant, lower values are an indication of stress (Hansatech Instruments Ltd 2010).

The other parameter of interest is the performance Index (PI) which uses three stages of the fluorescence process to give an indication of plant vitality.

Stage one can be described in terms absorbed excitation energy; The fraction of reaction center chlorophyll molecules (excited) relative to the total number of excited chlorophyll molecules. Stage two is the maximum yield of primary photochemistry (F_v/F_m) and finally stage three which is the probability that a trapped exciton moves an electron into the electron transport chain (ETC) beyond the primary electron acceptor (Q_a^-) (Strasser et al. 2000). PI is described

by Yunus et al. (2000) as an internal force of the sample to resist external constraints. PI is more sensitive to stress than F_v/F_m (Hermans et al. 2003) and was used in the analysis to aid interpretation of plant health.

Data from the beginning and end of the sample period was analysed using general linear models (GLM), as for growth parameters and A_n and any interval plots or barcharts shown include means $\pm$ 95 % confidecne intervals (CI).

Chapter 3

Results of Environmental Parameters.

Table 3.1: Soil characterisation showing; moisture content, total organic carbon (TOC), total Nitrogen (TN), mineral N (MN), available potassium (AK) and available phosphorous (P) of soils, and atmospheric NO_2 at each site (Upland site: Dartmoor Prison Farm (DPF), Rural sites Broadley (B) and Dartington (DT), Urban sites: Plymouth city allotments, Digginit(D) and Laira(L))

Soil characterisation and atmospheric NO_2 concentrations

ENVIRONMENT	SITE	pH	wet mass H_20	TOC	TN	MN	P	K	NO_2
			%			$g\ kg^{-1}$		cmolc Kg^{-1} K^{+} *	$\mu g\ m^3$
UPLAND	DPF	4.77	34.39	8.12	2.79	0.01	0.15	0.59	0.16
RURAL	B	5.63	29.35	5.05	2.95	0.02	0.04	0.96	0.18
	DT	5.18	28.64	5.04	3.17	0.01	0.12	1.43	0.27
URBAN	D	6.37	23.74	6.88	3.33	0.02	0.21	1.18	0.61
	L	5.99	19.45	3.60	1.93	0.01	0.08	0.76	0.76
ALL POTS	POTS	6.41	49.65	13.20	7.52	0.07	0.04	4.32	**

* centimoles of exchangeable K cations per kg soil
** all pots including control site pots

3.1 Soils.

In general the pots had the highest soil moisture content and TOC. Macronutrients were also high with pots having highest TN, AP and AK present. This was expected as potted plants were potted using both soil and compost mix with added slow release fertilizer. Despite this the peaty top-layer found at the DPF site on the high moor also has relatively higher macronutrients and organic matter content, however the other sites although being allotment sites have had no compost added for this particular research which shows in the relatively low levels of TOC and macronutrients present (Table 3.1).

3.1.1 Soil Moisture.

Using equation 2.3, the wet mass percentage (%) of water was found (Figure 3.1a). As expected pots and upper layer of Dartmoor Prison Farm (DPF) had the highest wet mass % (49.7% &

47.5% respectively), due to the organic matter content present. The driest sites were the urban sites, with all sites and all layers under 30% water content. Laira, had the lowest overall water content of 19.5% (average of both layers) (see Figure 3.1a).

3.1.2 Soil pH.

All sites had slightly acidic soils as suggested by the Cranfield University Soil-scape viewer. DT had the highest acidity at pH 4.25 in the upper layer and pH 5.28 in the lower layer (Figure. 4) DPF was also acidic with lower layer at pH 4.81. The rural site B and the urban sites at D and L were slightly less acidic with upper layers of around pH 6. Overall acidity was greatest at the rural sites DT (pH 4.8), DPF (pH 5.2), B (pH 5.6), and slightly higher at the urban sites FM (pH 5.7), L (pH pH6) and D (pH 6.4) (see Figure 4 for pH info on separate layers) (see Figure 3.1b).

3.1.3 Total Organic Carbon Content (TOC).

TOC was greatest in pots (13.2%) (as expected). The top layer of soil at DPF contained higher amounts of TOC (12.47%) while all other sites were relatively lower (Figure 4). In all cases the 10-20cm soil layer contained much lower levels of TOC this was especially true for the two rural sites B and DT and one urban site at L. The % TOC was less than 4% for all rural sites in this layer only 2.94% for L. The urban sites D and FM had over 5% TOC which is around the average % for the top layer of a loam soil high in fertility (Tan 2005) (see Figure 3.1c).

3.1.4 Nitrogen.

Total Nitrogen (TN).

TN levels were highest in pots (7.52 $g\ kg^{-1}$) (as expected) with the urban site Digginit D and all the rural sites containing between 3.4 and 3.6 $g\ kg^{-1}$ of TN, only the urban sites at FM and L having less than 2 $g\ kg^{-1}$ of TN in upper layer of soil (see Figure 3.1d). All lower layers had smaller amounts of TN (generally less than 2 $g\ kg^{-1}$). Figure 3.1d shows the relationship between TOC and TN (TN relies on the presence of organic matter in order to be released) (see Figure 3.1d).

3.1.5 Mineral N (MN).

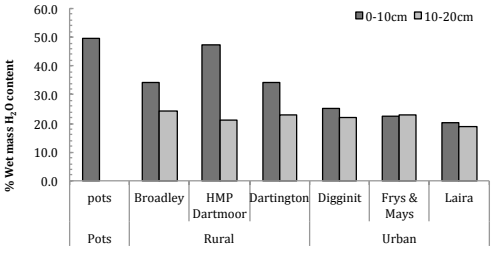
MN is usually around 2-3% of organic N (Tan 2005). However for all sites mineral N was low (less than 1% for topsoil and less than 0.5% for lower layers). Again pots had the highest mineral N however DPF and DT were the lowest (0.2% and 0.4% respectively) (see Figure 3.2c for soil N shown as a percentage of TN).

3.1.6 Available P (Bray 1 Extraction).

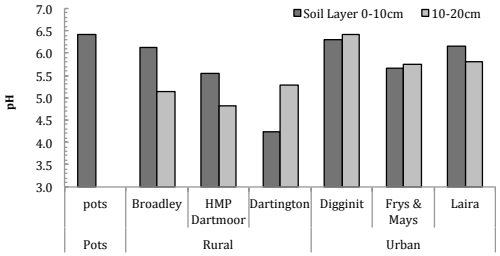
Phosphorous was actually low in the pots compared to the upper layer at DPF, D and DT. Lower layers were also high in P at the latter two sites perhaps a result of past management (see Figure 3.2a).

3.1.7 Available pottasium K (AK).

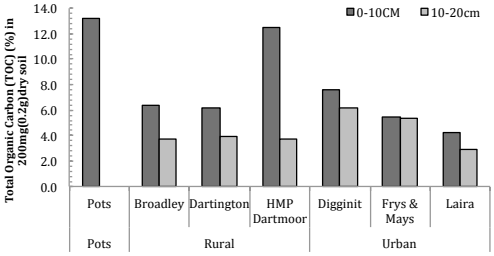
AK was highest in pots and between the planted sites top layer of soil contained the higher AK fraction, especially in the peaty topsoil of DPF (1.8 $mol\ kg^{-1}\ K^{+}$). Overall AK was low across soil layers and sites (see Figure 3.2b).



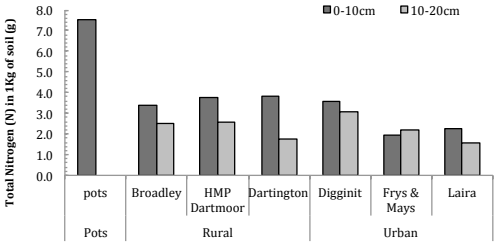
(a) Soil moisture: Wet mass H_2O content %



(b) Soil pH

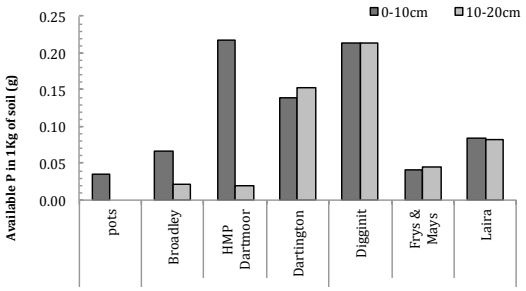


(c) Total organic carbon content

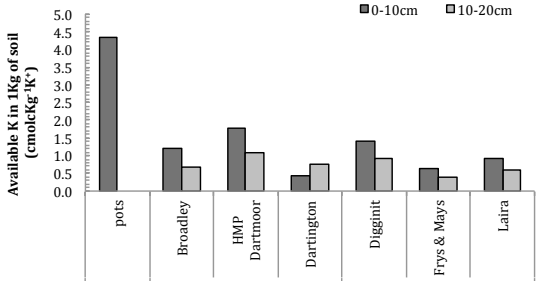


(d) Total Nitrogen (TN) in 1 Kg of soil (g)

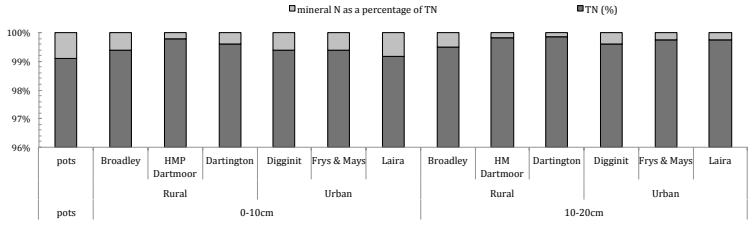
Figure 3.1: Soil moisture, pH, total organic carbon (TOC) and total nitrogen (TN) at each site (Upland site: Dartmoor Prison Farm (DPF), Rural sites Broadley (B) and Dartington (DT), Urban sites: Plymouth city allotments, Digginit(D) and Laira(L)), samples taken from 0-10cm and 10-20cm soil depths.



(a) Available P

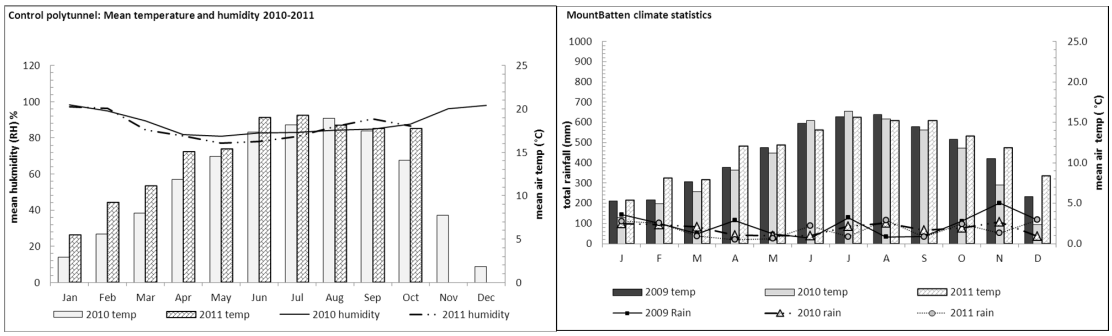


(b) Available K

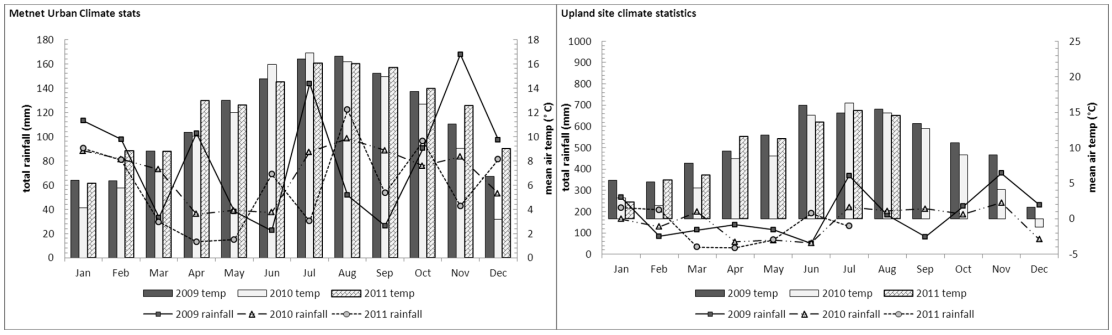


(c) Mineral N as a % of TN

Figure 3.2: Soil phosphorous (P), potassium (K) and mineral Nitrogen (MN) found in soil samples at 0-10cm and 10-20cm soil depths at each site (Upland site: Dartmoor Prison Farm (DPF), Rural sites Broadley (B) and Dartington (DT), Urban sites: Plymouth city allotments, Digginit(D) and Laira(L)).



(a) Control *(b)* Rural



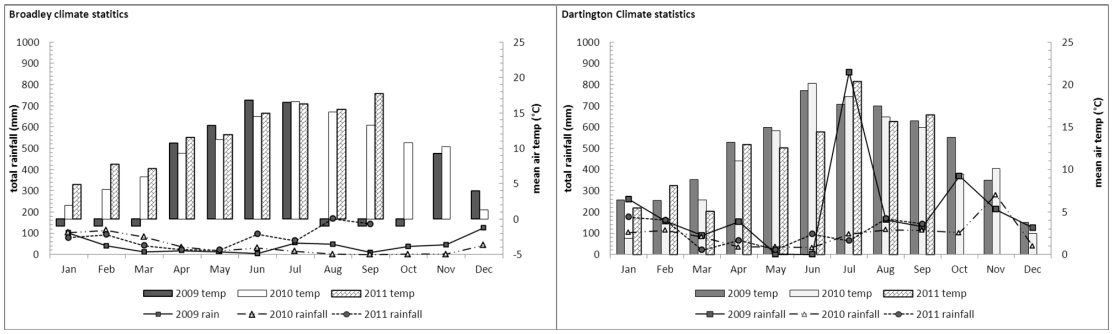
(c) Urban *(d)* Upland

Figure 3.3: Climate statistics for generic rural site (Mount Batten Weather Station, Plymouth), urban site (University of Plymouth, Metnet) and the Upland site (Environment Agency rain gauge and independent ibutton temperature loggers): bars show mean monthly temperatures and lines monthly rainfall totals for years 2009-2011

3.2 Climate Statistics.

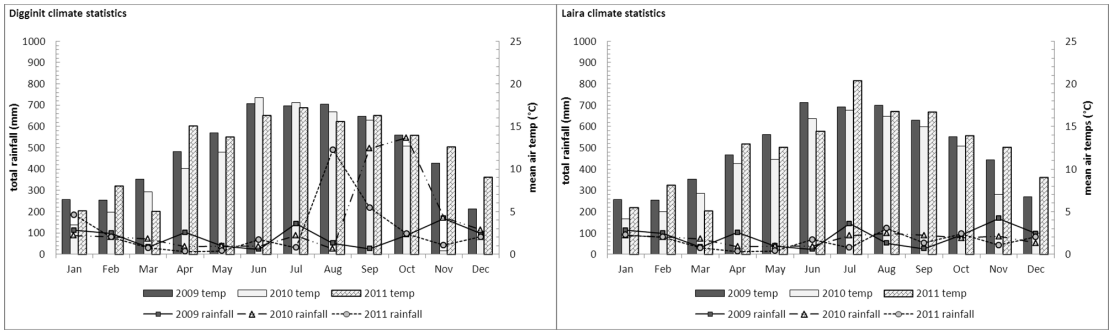
Figures 3.3 and 3.4 give mean temperatures and rainfall totals for each month in 2009-2010. There are climate statistics for each site complied from data loggers and rain gauges on site and also reference data for urban sites from the University of Plymouth's 'Metnet' weather station and the Met office data from Mountbatten station (situated as a reference for rural sites).

Mean temperatures were lower in 2010 than the other two years by about $1°C$ for all sites, with the upland site DPF having the lowest temperatures of $8°C$ in 2010 and $10°C$. the rural site Broadley, situated on the east Dartmoor fringe, had mean annual temperatures $1°C$ warmer



(a) Broadley

(b) Dartington



(c) Digginit

(d) Laira

Figure 3.4: Climate statistics for individual sites (Upland site: Dartmoor Prison Farm (DPF), Rural sites Broadley (B) and Dartington (DT), Urban sites: Plymouth city allotments, Digginit(D) and Laira(L)); bars show mean monthly temperatures and lines show monthly rainfall totals for years 2009-2011

in line with the Mt Batten Weather station with all other sites warmer on average by $2°C$. Mean temperatures were highest in June and July with rural site DT having the highest summer temperatures(maximum monthly mean $18.8°C$), followed by the urban sites, L ($18.2°C$) and D ($17.5°C$). Lowest temperatures occurred between December to February the lowest being at the upland site DPF ($0.51\ °C$) rural B ($2.9\ °C$) and rural DT ($3.4°C$) while the urban winter temperatures were between $5-6°C$ for these months (evidence for the urban heat island effect increasing winter averages at the urban sites, despite summer averages being higher at rural DT.

Rainfall was lowest at the rural site B as this site falls within the east Devon 'rain shadow' discussed by (Phillips and Mcgregor 2002; Phillips and Denning 2007), with less than 100mm of rain falling each month during 2009 and 2010 and annual totals of 500, 442 and 730 respectively for each year of the experimental period. Although upland DPF had higher overall rainfall during each year (2238, 1806 and 1090 respectively), the rural site DT seemed to have the most rainfall during July 2009 (over 800mm) and yearly totals of (2500, 1150 and 916 respective years) almost twice as much as the rural site B (see Figure 3.4 for more information). Rainfall data for the urban site L has been taken from the University Metnet data and yearly totals for this site were (980, 840 and 720) respective years.

3.3 Nitrogen Dioxide (NO_2).

The air quality objectives set out via government regulations through "Local Air Quality Management (LAQM)" and in relation to the Environment Act 1995 (Environmental Protection and Monitoring Unit 2009) give a target annual mean of 40 $\mu g\ m^3\ NO_2$. Stoke Village has been highlighted within the report as a key area of concern for NO_2 pollution and is situated in front of the 'Digginit' urban nursery site. Tavistock road, the main route into the centre from Stoke village is also a key pollution area and the both urban mature tree sites were situated near to this road (in particular the *Q. robur* site. Tavistock road diffusion analysis by Plymouth City Council regularly exceeds the AQO of 40 $\mu g\ m^3$, background concentrations which should be indicative of the nursery sites were between 16.9 and 17.8 in Plymouth (2008 data) (Environmental Protection and Monitoring Unit 2009). A biomass adjustment factor (BAF) was used to correct the results, using the local adjustment factor created by Plymouth City Council from

Table 3.2: Nitrogen dioxide (NO_2) levels recorded via diffusion tubes at each site and sites combined into environmental means (Upland environment: Dartmoor Prison Farm (DPF), Rural environment: Broadley (B) and Dartington (DT), Urban environment: Plymouth city allotments, Digginit(D) and Laira(L)).

		Monthly NO_2 per SITE ($\mu g^{-1}\ m^2\ NO_2$)						
Environment		URBAN			CONTROL	RURAL		UPLAND
Date	Site	FM	L	D	CS	DT	B	DPF
Jun/Jul		0.72	0.79	0.72	0.32	0.4	0.23	0.17
Jul/Aug		0.57	0.69	0.45	0.12	0.21	0.15	0.14
Aug/Sep		0.63	0.65	0.6	0.2	0.21	0.18	0.16
Oct/Nov		0.27	0.9	0.67	0.22	0.19	0.2	0.14
Annual monthly mean		0.55	0.76	0.61	0.22	0.25	0.19	0.15
Environment mean			0.64		0.22	0.22		0.15

2008 data, of 0.92 $\mu g\ m^3$.

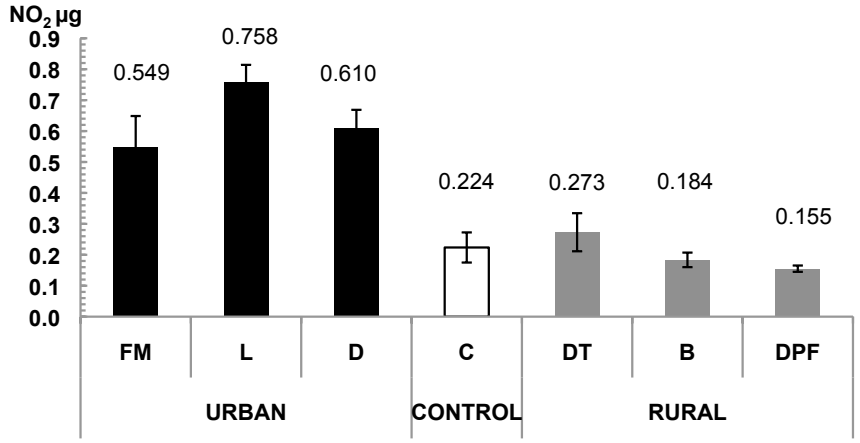


Figure 3.5: Annual monthly means for NO_2 diffusion tubes at each site (Upland site: Dartmoor Prison Farm (DPF), Rural sites Broadley (B) and Dartington (DT), Urban sites: Plymouth city allotments, Digginit(D) and Laira(L)).

Chapter 4

Photosynthesis and Chlorophyll fluorescence.

Field based photosynthesis measurements are useful tools for analysing productivity in particular with reference to carbon sequestration potential in trees. This chapter evaluates mean assimilation rates for both species at each of the sapling sites, stomatal conductance (Gs) and intrinsic water use efficiency ($iWUE$) as the ratio of net photosynthetic rate (A_n) and stomatal conductance to water vapour (Gs) and photosynthetic efficiency, using variable fluorescence over maximum fluorescence (F_v/F_m) as a measure of maximum efficiency of photosystem II, photochemical quenching and a newer 'performance index' (PI) which incorporates three phases of the photosynthetic process and is a measure of plant vitality. Parameters were measured on ten trees per species at each urban, rural and upland site, from two leaves in the upper canopy and two leaves in the lower canopy to establish if there were differences between sun and shade leaves. Measurements were taken between 8 am and 12 pm during each monthly sampling date. Light response curves were completed for each species and diurnal photosynthesis was also measured (methods described in the general methodology chapter 2. In control site saplings, net assimilation rates were higher in the deciduous oak, however the evergreen was able to increase assimilations rates in early Spring when temperatures were cool but above 5°C despite lowered efficiency. Effects of environment were site specific; at the upland site, *Q.ilex* experienced lower assimilation rates and reduced F_v/F_m during winter while in both species assimilation was high during the summer at this site but PI was lower throughout the year. The evergreen oak maintained mean assimilation rates of around 7 $\pm$ 0.4 $\mu mol\ s^{-1}$ throughout the year at rural site, DT, and urban site, D where average minimum temperatures were higher. In contrast, both species experienced stress at the rural site B and urban site L, and mean assimilation rates were lower during winter months in *Q. ilex*. However overall assimilation rates were higher in *Q. ilex* at these sites. In conclusion, although the

deciduous *Q. robur* has higher carbon assimilation rates and photosynthetic efficiency in fertile soils where water availability is high alongside higher average temperatures, those sites where soil fertility and water availability are low mean that the evergreen oaks water conservation strategy gives it an advantage. This advantage is increased at sites where mean minimum temperatures are higher and consequently *Q. ilex* winter assimilation rates remain high.

4.1 Introduction.

4.1.1 Photosynthesis.

Photosynthesis is the process by which plants utilise the energy of the sun to create biochemical reactions using CO_2 and H_2O molecules. These biochemical reactions form carbon compounds such as carbohydrates used by plant cells for respiration and growth (see Chapter 1 section 1.2.1). It follows that photosynthetic rate is directly linked to the assimilation of carbon minus the loss of carbon through cellular respiration (e.g. Lambers et al. 2008; Pallardy 2008); net photosynthesis (NP) is equal to gross photosynthesis (GP) minus respiration (R) (see equation 4.1).

$$NP = GP - R \tag{4.1}$$

(Whitmarsh and Govindjee 1999).

4.1.2 Respiration.

Respiration (R) is closely coupled with carbon assimilation rates and is controlled by the energy demand of the plant (e.g. Lambers et al. 2008). Shade plants therefore tend to have lower respiration rates than sun adapted plants (Givnish 1988). Shade leaves of red oaks (*Q. rubra*) had lower A_n, followed by lower R rate the following night, in response to reduced carbohydrate availability compared to upper sun leaves (Whitehead et al. 2004). Leaf R is generally suppressed in the light (Villar et al. 1995) although the reduction in CO_2 respired during daylight has been attributed to its re-use during photosynthesis (e.g. Delfine et al. 1999). R is also known to increase with rising temperatures however, in some plants, R has been shown to even-

tually decrease as thermal acclimation occurs (VanOijen et al. 2010). For example, both potted and field grown red and white oaks exposed to sudden temperature increases exhibited a rapid thermal acclimation (Bolstad et al. 2003). The same study also showed that red and white oaks could maintain maximum assimilation rates between 18 and 30°C, increasing A_n with increasing temperatures while R decreased, and therefore increasing the amount of carbon assimilated (Bolstad et al. 2003).

4.1.3 Diurnal rhythms.

Early studies on diurnal photosynthesis have found that tree species increase their photosynthetic levels throughout the morning reaching a peak before or around noon, depending on light levels and followed by a midday depression (Kozlowski 1971). Even earlier work showed that conifers on cloudy or overcast days increased A_n to a maximum at noon, A_n remained constant for an hour before gradually reducing, while on sunny days A_n increased quickly peaking between 9am and 12pm, decreasing before a second rise in late afternoon (Hodges 1967). More recently, red oaks, along with three other deciduous species showed a decline in A_n throughout the day (Bassow and Bazzaz 1998). For deciduous species such as oaks, second flush leaves tend to have higher mean A_n than first flush leaves (Masarovicova 1991). The same study found A_{max} in oaks tended to occur between 8 and 10am during the summer with a midday depression (Masarovicova 1991).

Closely coupled with diurnal and seasonal photosynthesis is photosynthetic efficiency, with many tree species experiencing a reduction in photosynthetic efficiency as temperature and light increases throughout the morning and in mid Summer. For instance, despite a humid summer during data collection, A_n and Gs decreased during a warm summer along with a decrease in F_v/F_m (Peñuelas et al. 1998). Moreover, F_v/F_m levels increased as temperatures dropped, with the reduction in photosynthetic efficiency attributed to a down regulation of photosynthesis during warmer temperatures (Peñuelas et al. 1998).

In situations where a decrease in photosynthetic efficiency, is not followed by immediate recovery after stress relief, reductions in F_v/F_m and associated chlorophyll fluorescence parameters may indicate photo-inhibition. Photo-inhibition can occur when shade adapted plants are sub-

jected to sudden high light environments, coupled with either high or very low temperatures depending on the bio-climatic envelope and plasticity of the species being studied (see Alves et al. 2002). In general *Quercus* spp. tend to exhibit high phenotypic plasticity, in particular, *Q. ilex* has been shown to represent a complex species (Barbero et al. 1992) and has developed phenotypes that can withstand temperature extremes of $+50^{\circ}$C to -10°C (Gimeno et al. 2009).

4.1.4 Water use efficiency.

Water use efficiency is generally measured as the ratio between net photosynthesis and transpiration (A_n/E), however, as *Gs* and transpiration are directly related through stomatal function, water use efficiency can also be assessed using the ratio of A_n to *Gs*, known as intrinsic water use efficiency ($iWUE$). $iWUE$ is a representation of internal CO_2 concentration and its effect on water use efficiency (Linderson et al. 2012). This parameter has been used in the literature as a substitute for WUE, as E is affected by humidity and leaf temperature and subsequently leaf to air vapour pressure difference but is also directly related to *gs* (Stokes et al. 2010). High levels of leaf level internal CO_2 can be an indication of low WUE. Therefore it is possible for a plant to maintain high levels of assimilation rates while still reducing $iWUE$. $iWUE$ may increase with increasing light (Ponton et al. 2002) although this effect is generally lower for less drought tolerant species and is likely to be due to genetic differences within related oaks (Ponton et al. 2002).

4.1.5 Species and environment.

Some authors suggest there will be a change from deciduous to evergreen species with future climate change (e.g. Both and Bruggemann 2009), yet others document the succession of beech (*Fagus sylvatica*) into oak dominated forests (Rohner et al. 2012) and in drier areas the replacement of Beech dominated forests to oak (Ogaya and Penuelas 2007*b*). Furthermore, *Q. ilex* is extending its range further north into central Europe and *Q. ilex* (sub sp. *ilex*) is already found along the Atlantic coast of France as far as Brittany (Barbero et al. 1992).

Research also shows that *Q. ilex* is able to tolerate areas where pollution is a problem better than *Q. robur*. One open top chamber study on ozone (O_3) effects on gas exchange and stomatal

conductance (*Gs*) showed that *Q. ilex* was able to maintain higher *Gs* levels as O_3 concentrations increased while *Q. robur*, having larger, thinner leaves, showed signs of stomatal blockage and yellowing of leaves (Calatayud et al. 2011). In contrast, both a deciduous and evergreen oak were shown to have beneficial effects on urban temperatures and carbon sequestration potential in Rome (Gratani and Varone 2006).

To summarise, *Q. robur* enjoys maximum productivity in lowland Southern England and is normally replaced by the European deciduous Sessile oak (*Q. petrea* on poorer, upland soils (Broadmeadow and Matthews 2003). However, evidence of *Q. robur's* increasing productivity has been noted in Wistmans wood, Dartmoor since mid twentieth century (e.g. Simmons 1965). Indeed, the UK Forestry Commission has suggested *Q. robur* will replace the European Sessile oak *Q. petrea* as the dominant species in the Southwest with future climate change (Broadmeadow 2004; Broadmeadow et al. 2005). On the other hand, *Q. ilex* already has established populations in mountainous regions of the Iberian peninsula, and can tolerate climatic extremes up to an altitude of around 1500m (Reille and Pones 1992) despite exposure, poorer soils and cooler temperatures reducing productivity in these environments. The Southwest UK upland environment, although only reaching altitudes of $400-600masl$ at most, is subject to poor soils, irregular frosts and long periods of wet weather. Carbon assimilation rates and photosynthetic efficiency of both species were measured on monthly basis in such an upland environment while also comparing them in urban and rural locations within the Southwest UK, to aid comparison of carbon sequestration potential in each species.

In this chapter, section 4.3 and section 4.4 introduce light saturation curves, diurnal rhythms and average dark respiration for each species at the control site where nutrient levels and water availability were good. Section 4.5 covers monthly photosynthesis and photosynthetic efficiency in well watered control trees over a year. The results of a small drought experiment taken on the three year old control site saplings is also shown in this section. Section 4.5 first compares differences between potted (P) and field grown (G) saplings at each site (section 4.5.4) then gives results on seasonal photosynthesis (section 4.5.5) and efficiency at each site and comparisons between rural, urban and upland environments are made (where differences between

soil conditions have been analysed control site data has not been included due to there being no field planted saplings at this site and consequent unbalanced design). Section 4.5.6 covers the differences between sun and shade leaves in the upper and lower canopy (not all saplings developed sun and shade leaves due to lack of foliage). Parameter units are introduced but not used throughout the chapter, please refer to the glossary for these units.

4.2 Respiration.

4.2.1 Methods.

Six saplings of each species had photosynthetic rate measured using the LCi portable IRGA. Four mature leaves were used per tree and for *Q. ilex*, two new leaves per tree were also measured. Data for daytime assimilation rates were compared with respiration rates for the same day and results analysed using ANOVAs with assumptions for normality met (Levenes test, $P > 0.05$). It is acknowledged that this may not be an accurate indication of dark respiration rates during the day due to the temperature control of respiration (Bolstad et al. 2003) however this is an indication of differences in respiration between species.

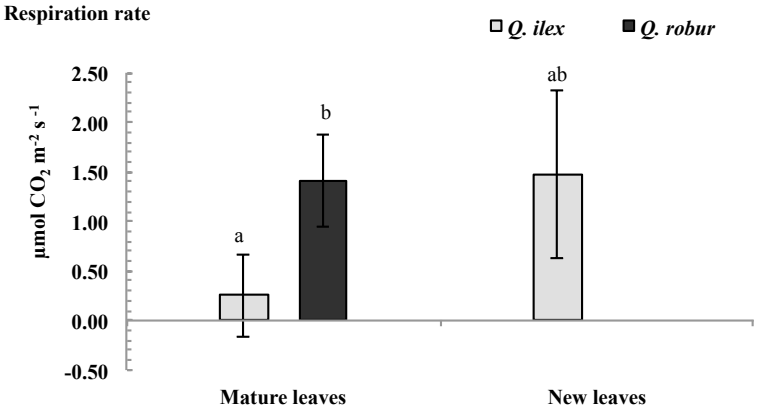
4.2.2 Results.

Q. robur had higher mean net photosynthetic rates than *Q. ilex* during the day. During the night net respiration was also significantly higher for *Q. robur* than *Q. ilex* (-1.41$\pm$ 2.32; -0.25$\pm$ 2.11 respectively) ($F=13.08_{(1,190)}$, $p<0.001$). For newly developed *Q. ilex* leaves, respiration was approximately twice the value of mature *Q. ilex* leaves (see Figure 4.1).

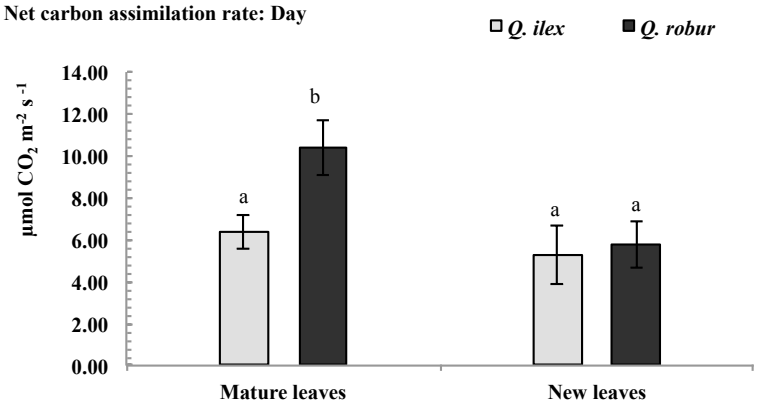
4.3 Light Saturation Curves.

4.3.1 Methods.

Light response curves were created for both species using well watered, two year old pot grown saplings taken from the control site. Trees were removed from the polytunnel during the experiment and averages taken from three leaves per tree from the upper and lower area of foliage (this was to ascertain if there was differences between sun and shade leaves). Photosynthetically active radiation (PAR) levels were set using neutral density filters, with the ADC light



(a) Mean respiration rate



(b) Mean daylight assimilation rate

Figure 4.1: a). Mean respiration rate for each species recorded overnight June 2010 and, b) day-time assimilation rate for daylight hours of the same day.

unit (supplied with a 'dichoric' 20 watt bulb). The maximum PAR leaf level obtained by the light unit was around 550 PAR. Where possible leaves were exposed to natural light in order to obtain higher PAR levels (see methods section 2.3.6 on page 29). Curves were produced in August 2010 for both species and in February and again in April 2011 for *Q.ilex*.

4.3.2 Results.

In August 2010 when mean temperatures were approximately 15-16 $^{\circ}C$, *Q. ilex* shade leaves were light saturated at around 350 PAR at 6.5 $\pm$ 0.5 and *Q. robur* shade leaves saturated at similar light levels but assimilation rate was lower at 5.8 $\pm$ 1.08 (see Figure 4.2a on page 51). *Q. ilex* sun leaves saturated at approximately 500 PAR with assimilation rate reaching 8.8 $\pm$ 1.06 and *Q. robur* reached a maximum of 7.8 $\pm$ 0.918 at the higher PAR of 600 (see Figure 4.2). Light response of *Q.ilex* in April 2011 at *Q. robur* bud burst, showed that *Q. ilex* reached light saturation at 8.5 at 700 PAR when mean temperature was around 11 $^{\circ}C$, during this experiment *Q. ilex* was able to maintain maximum rates as light increased to 2000 PAR (see Figure 4.2). However, light response in February 2011 when mean temperatures were around 5$^{\circ}C$ was much lower (Figure 4.3).

4.4 Diurnal Rhythms.

4.4.1 methods.

Data was collected in July 2009 to find natural diurnal rhythms. photosynthesis measurements were taken in sets of two leafs per three trees per species between 5:00 and 17:00. The averages of each set of data were used for analysis. Results were then pooled according to the nearest 15 minute interval, between 5:00 and 17:00. No distinction between upper and lower canopy were made at this growth stage. For diurnal rhythms using the light unit, data was collected during July 2010 in sets of 3 trees per species on two upper and two lower leaves plus two new flush leaves where possible. Data was collected between 8:30 17:00 and analysed as before using averages of sets.

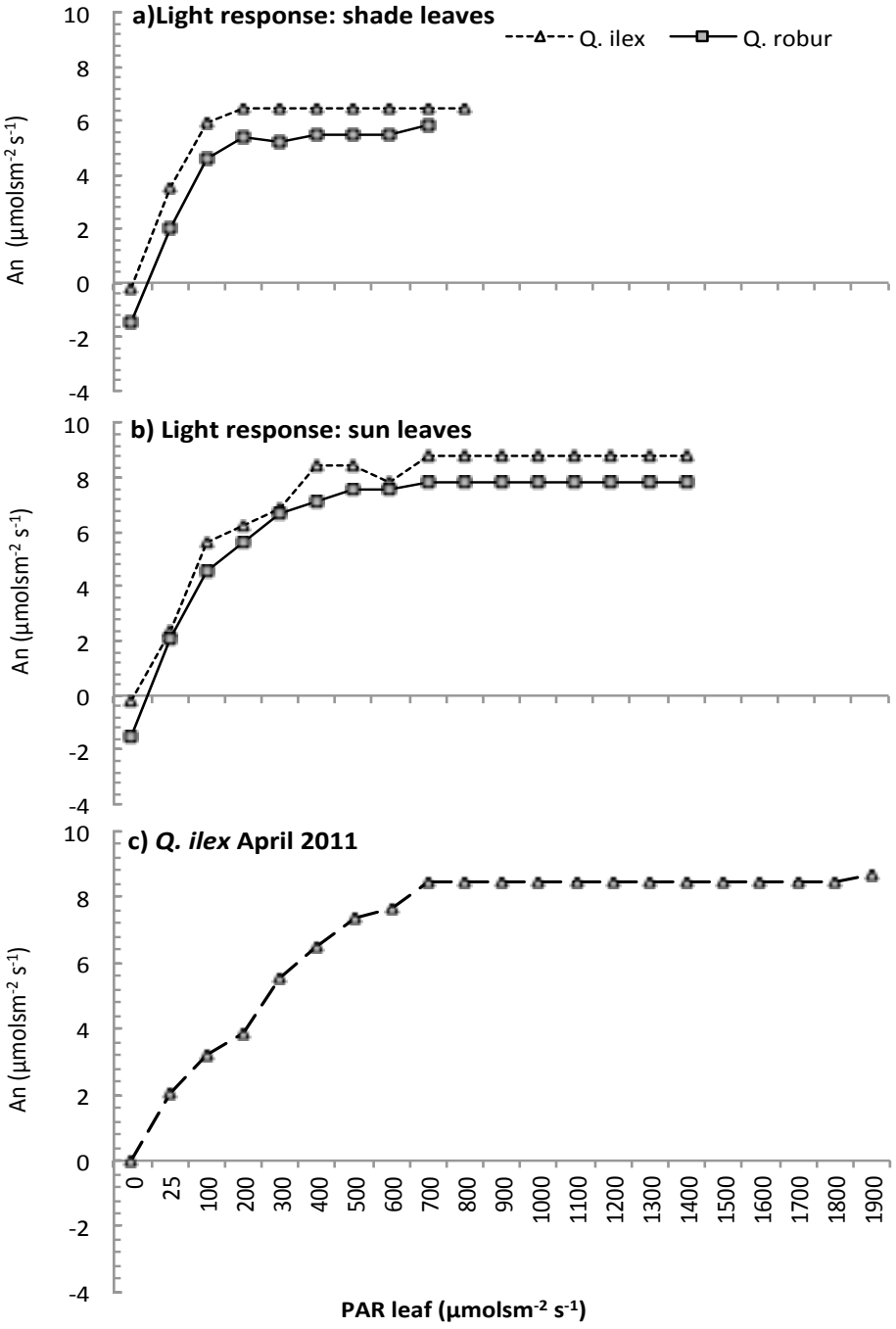


Figure 4.2: Light response curves for a) shade, b) sun, leaves for each species taken in August 2010, and c) *Q. ilex* April sun leaves

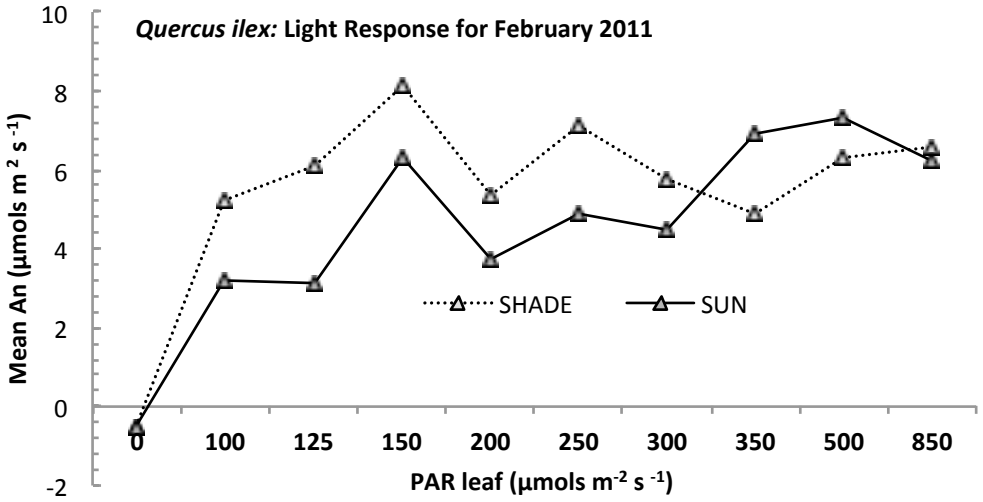


Figure 4.3: Light response curves for *Q. ilex* winter sun and shade leaves (February 2011).

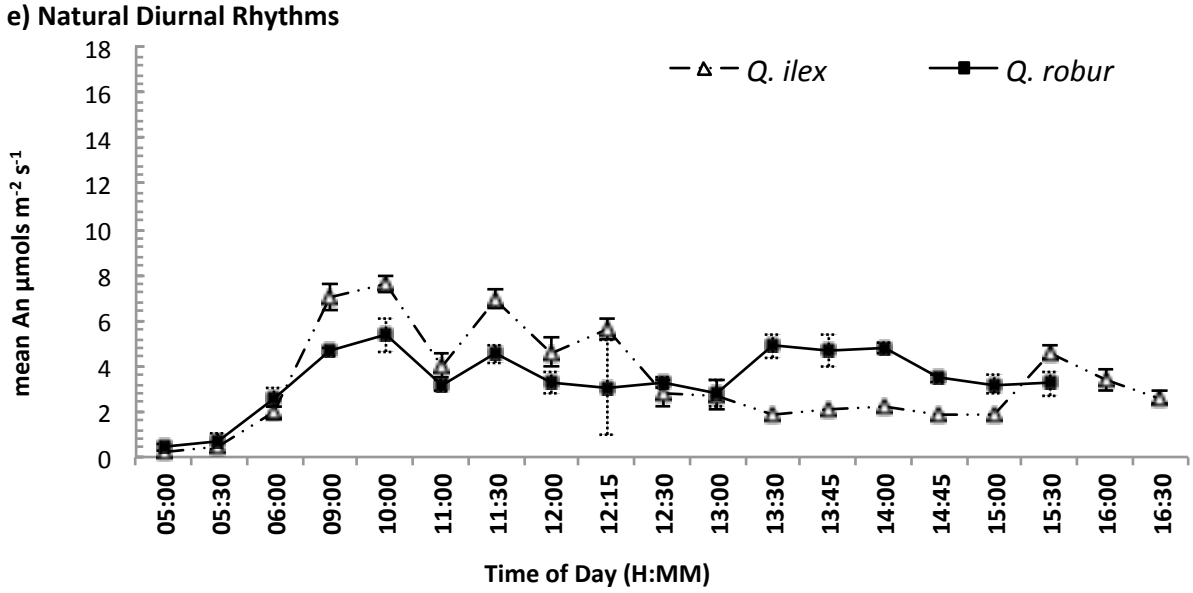


Figure 4.4: Diurnal rhythms under natural light for *Q. ilex* (open triangles) and *Q. robur* (closed squares). (means ± 95 % CI), data taken over two consecutive days in July 2009 on two leaves from three trees per species. Average temperatures for July 2009 were 19.1 ± 0.39 and for the two day period July 10-11th 2009 16 ± 1.04 and days were clear and sunny.

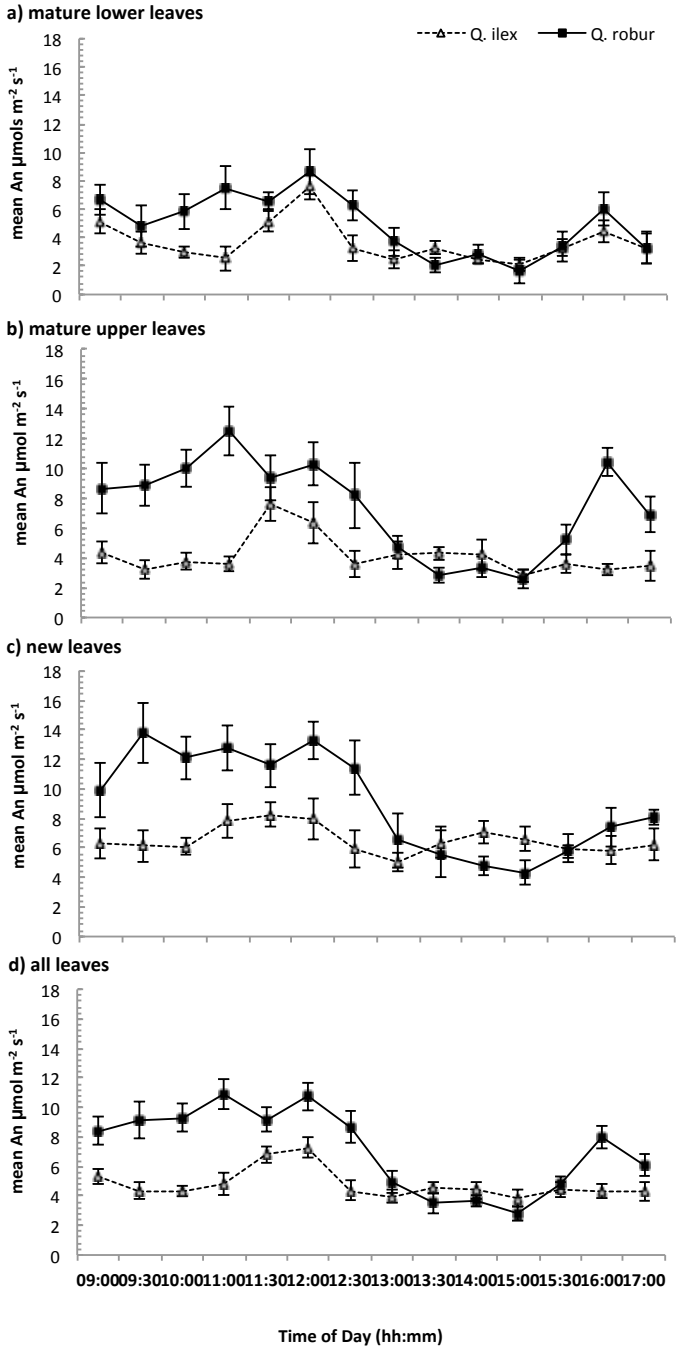


Figure 4.5: Diurnal rhythms at fixed PAR 550 $\mu mol\ s^{-1}\ m^2$ for, a) lower, b) upper, c) new and d) all, leaves for each species (means $\pm$ se shown per time interval)

4.4.2 Results.

For natural diurnal rhythms, *Q. robur* had lower assimilation rates than *Q. ilex* with maximum photosynthesis(A_{max}) of around 5.5 occurring at 10:00 and again at 13:30. In contrast, *Q. ilex* reached maximum assimilation rates closer to 7.5 but these quickly fell after midday and remained low until the end of data collection (see Figure 4.4 page, 52).

Diurnal rhythms taken using the light unit however showed *Q. robur* to have higher assimilation rates and mean A_n reached close to 11 (see Figure 4.5 d). Mature upper leaves *Q. robur* obtained a maximum A_n, 5 higher than *Q. ilex* by mid morning 11-11:30 and continued to maintain these rates until the mid day decrease around 13:00 (see Figure 4.5. Although both species experienced a mid-day decrease in assimilation rates, *Q. robur* increased its rate again in the late afternoon.

A similar pattern is followed with lower leaves, but with lower mean assimilation rates (see Figure 4.5). New leaves have the greatest assimilation rate and seem to reach higher rates earlier in the day with *Q. robur* having reached maximum rates by 09:30. Mean rates did not decrease again until 13:00 but again *Q. robur* increased mean A_n to 7-8 after 16:00 (see Figure 4.5 page 53).

4.5 Species and Environment.

4.5.1 Methods.

See chapter 2, section 2.3.6 for sampling protocol for monthly photosynthetic parameters. For the drought experiment three 3 year saplings of each species were subjected to two weeks of drought and measurements of photosynthetic parameters were taken on four leaves of each tree.

Results shown are; monthly assimilation rates and stomatal conductance of CO_2 (A_n and Gs) and photosynthetic efficiency (F_v/F_m and PI) for control saplings. Results from the drought experiment show A_n, Gs, sub-stomatal CO_2 and transpiration (E) data, but unfortunately no chlorophyll fluorescence data was taken for this experiment. Sections 4.5.4 gives any significant differences between pots and ground planted saplings in order to indicate how fertility affected each species. Seasonal differences between species and site or site characteristic (rural,

urban or upland) are shown, and finally section 4.5.5 gives species differences in leaf position in canopy at each site to show differences in sun and shade leaves where relevant (not all saplings developed thick enough canopies for sun and shade differentiation). Intrinsic water use efficiency was also measured using A_n / Gs and this was analysed between species, site and soil condition.

4.5.2 Results: Monthly Photosynthesis and Chlorophyll Fluorescence.

In well watered control saplings, Both species reached maximum assimilation rates in Spring (8 ± 1.6), however *Q. ilex* attained this maximum earlier as *Q. robur* leaves were still developing (Figure 4.6). However, by the end of May, *Q. robur* reached comparable A_{max} and maintained these higher assimilation rates throughout the summer months until senescence towards the end of October when mean air temperatures dropped (Figure 4.6). As temperatures decreased *Q. ilex* A_n increased again as *Q. ilex* took advantage of the milder Autumn weather. Chlorophyll fluorescence values remained high in *Q. ilex* despite its down regulation of assimilation rates during the hotter summer months, however both species showed reduced *PI* during the colder months: *Q. robur* as leaf senescence began in Autumn and for *Q. ilex*, during the Winter months even when A_n was high (Figure 4.7).

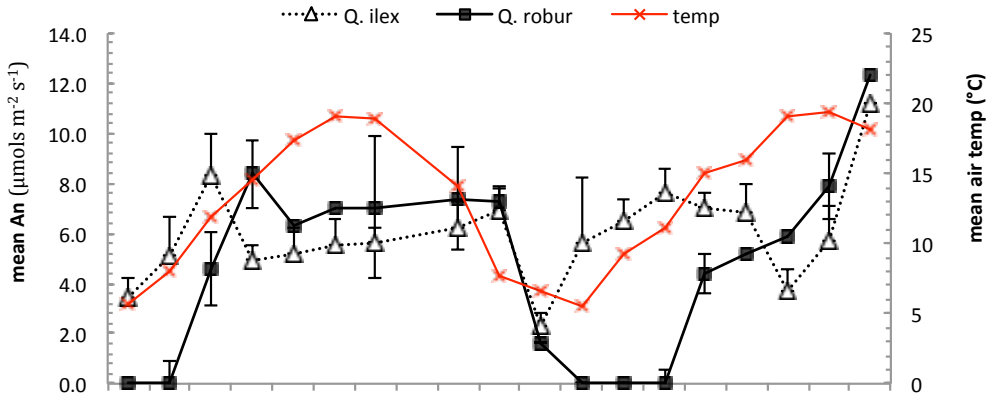
4.5.3 Results: Drought.

Table 4.1: ANOVA table showing effects of treatment (drought or well-watered saplings) on mean photosynthetic rates (A_n $\mu mol\ s^{-1}\ m^{-2}$), sub-stomatal CO_2 (Ci vpm), transpiration rates (E $mmol\ m^{-2}s^{-}$) and stomatal conductance of CO_2 (Gs $mol\ m^{-2}\ s^{-1}$) showing F statistics and p-values

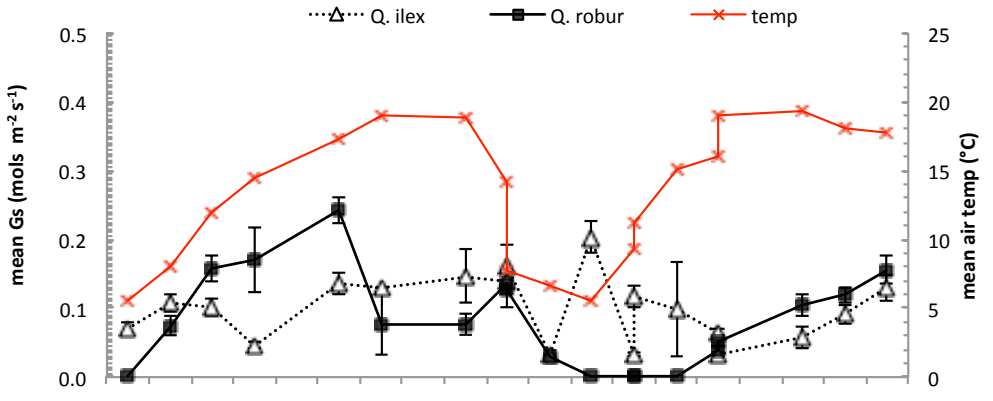
Results of LM: ANOVA table

	Physiological parameters								
		A_n		Ci		E		Gs	
Factors	DF	F	P	F	P	F	P	F	P
SPECIES	1	8.49	0.005**	4.64	0.04*	11.78	0.001***		ns
TREATMENT	1	46.42	0.0***		ns	120.13	0***	50.65	0***
SPECIES*TREATMENT	1	6.46	0.01**	4.43	0.04*		ns		ns
Error	64		ns		ns		ns		ns
ADJ R^2 (%)		45.62		11.44		65.12		41.84	

Q. ilex had around 50% lower mean A_n, in well watered saplings, than *Q. robur* (see Figure 4.8a). However, both species had similarly reduced A_n with drought (1.7 - 2.0 $\mu mol\ m^{-2}\ s^{-1}$)

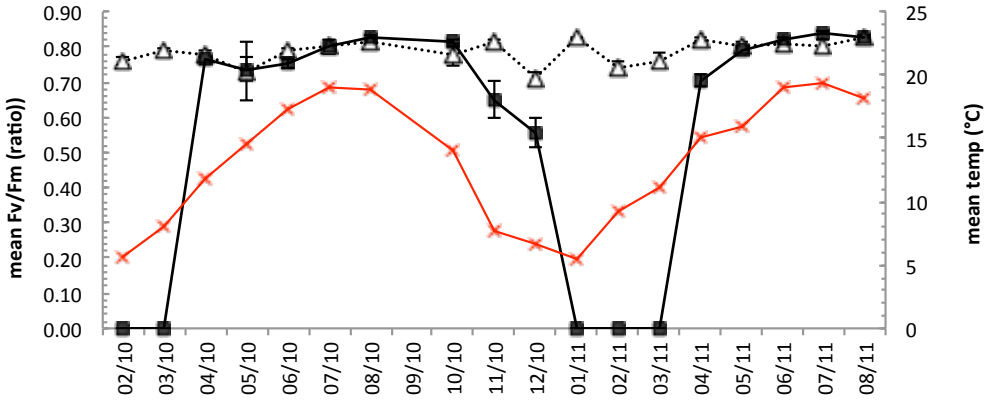


(a) Monthly assimilation rate (A_n).

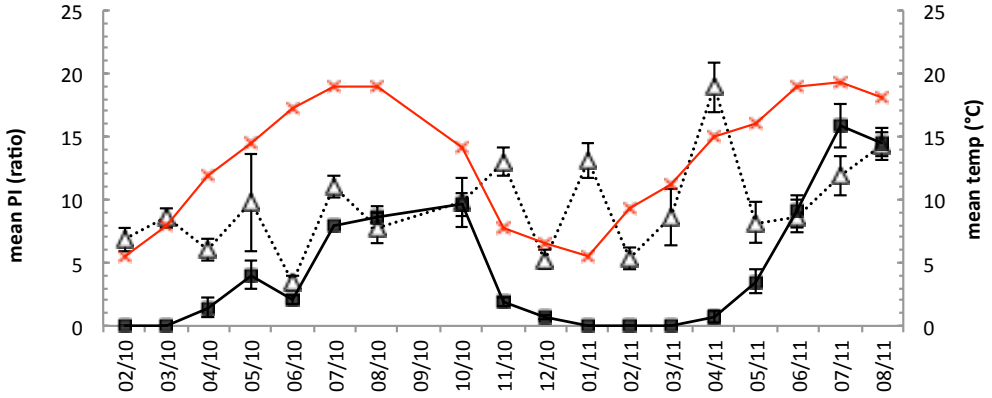


(b) Monthly stomatal conductance (*Gs*).

Figure 4.6: a) Monthly assimilation rate (A_n) and b) Monthly stomatal conductance (*Gs*) for well watered control site saplings, shown with mean monthly temperatures (°C) (means ± 95% CI).



(a) Monthly variable fluorescence over maximum fluorescence ratio (F_v/F_m)



(b) Monthly performance index (PI) for well watered control site saplings.

Figure 4.7: Monthly a) Maximum photochemical quenching shown by (F_v/F_m) and b) Sapling vitality shown by performance index (PI) for well watered control site saplings, shown with mean monthly temperatures (°C) (means $\pm$ 95% CI).

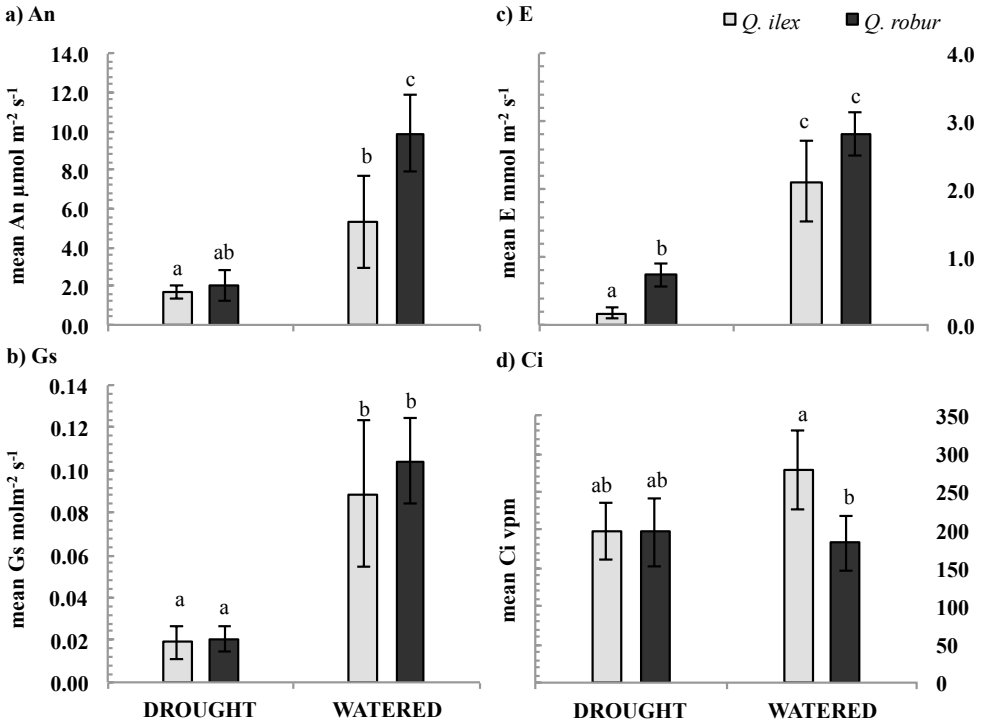


Figure 4.8: Differences in photosynthetic parameters for well watered saplings and saplings that had been subjected to two weeks of drought conditions), a) mean net assimilation rates (A_n), b) mean stomatal conductance (Gs), c) mean transpiration rate (E) and d) mean sub-stomatal CO_2.

(see 4.8 and 4.1 on pages 58 and 55.

Mean *Gs* was 10% lower for drought saplings in both species compared to well watered values. Despite similar *Gs*, *E* was higher in *Q. robur* saplings during drought and *Ci* was also lower in well watered *Q. robur* saplings despite similar decreases with drought for both species (see table 4.1, page 55 and Figure 4.8, page 58).

4.5.4 Results: Differences between potted and field grown saplings

Although there were significant effects from the urban, rural and upland environment, these effects were site specific and

Data for net assimilation rate A_n and photosynthetic efficiency parameters F_v/F_m and *PI* had equal variances using Levenes test for any continuous distribution (p>0.05) for all factors. However, *Gs* and *PI* data were skewed, therefore data were log transformed to equalise variances (p>0.05). Mean assimilation rates were not affected by condition (table 4.2), however an interaction with site showed that urban saplings had higher A_n in (this was more obvious in *Q. ilex* where A_n ranged between 3.5 - 5.5 $\mu mol\ m^{-2}\ s^{-1}$ in potted saplings and 5.5 - 7.2 $\mu mol\ m^{-2}\ s^{-1}$ in field grown saplings) whereas potted were greater than field grown in rural saplings. *Gs* was higher in potted for *Q. robur* although conversely in *Q. ilex* field grown had higher rates of *Gs*. Photosynthetic efficiency was not different between potted and field grown in *Q. ilex* however, in *Q. robur* saplings potted was always higher than field grown (most obvious at the upland site where F_v/F_m varied from 0.7- 0.78 in field grown planted saplings to 0.8-0.82 in potted). All parameters measured had a three-way interaction between soil condition, species and site and showed that carbon assimilation rates and photosynthetic efficiency parameters were higher in pots than field grown saplings, however at the urban site (L) the opposite was true. F_v/F_m was higher in pots than field grown saplings for *Q. robur*, whereas for *Q. ilex* the opposite was true. (Table 4.2 shows effects and interactions between soil condition, species and site for photosynthetic parameters).

Table 4.2: ANOVA table shows significant differences between sites, species and soil condition (where Condition = potted (P) or field grown (G) saplings). (F statistics and p<0.05 '*', p<0.01 '**', p<0.001 '***' and p>0.05 'ns'). Data was analysed using application of general linear models on entire annual dataset.

ANOVA Table: Differences between field grown and potted saplings and interactions with site and species shown

Physiological parameters		A_n		LogGs		iWUE		FV/FM		log(PI)	
FACTORS	Df	F	sig.	F	sig.	F	sig.	F	sig.	F	sig.
CONDITION	1	0.0	ns	37.7	***	25.9	***	2.4	ns	0.1	ns
SPECIES	1	12.0	***	118.5	***	113.1	***	4.3	*	560.1	***
SITE	4	7.7	***	68.4	***	43.0	***	122.5	***	123.0	***
CONDITION:SPECIES	1	0.5	ns	15.4	***	1.9	ns	5.6	*	5.9	*
CONDITION:SITE	4	6.1	***	15.4	***	10.9	***	13.7	***	19.7	***
SPECIES:SITE	4	61.8	***	34.4	***	5.2	***	56.9	***	60.1	***
CONDITION:SPECIES:SITE	4	5.3	***	12.7	***	2.9	*	5.0	***	10.5	***
Residuals IRGA	3229										
Residuals PEA	5287										

Sapling $iWUE$.

Effects on $iWUE$ were site specific (Table 4.2). $iWUE$ was higher in *Q. ilex* than *Q. robur*, generally higher in pots than field grown saplings except at the urban site, L, where field grown saplings had significantly higher $iWUE$ than pots. For field grown saplings, $iWUE$ was also significantly higher at the urban site L than all other sites in both species (74-86 $\mu mol\ CO_2$ / H_2O^{-1} compared to 40-50 $\mu mol\ CO_2$ / H_2O^{-1} compared for all other sites and species combinations).

4.5.5 Results: Seasonal Leaf Physiology for Species and Environment.

Phenology.

Forestry Commission phenology research gives phase 2 as bud formation and greening, phase 3 as leaf unfurling and visible leaf form and phase 4 as fully expanded leaves (Forestry Commission 2012*a*). Using this information and 20th April 2010 as a reference date (this being an average date for bud burst in Southwest as shown by the Forestry Commission bud-burst map (Forestry Commission 2012*a*) *Q. robur*), bud-burst at the different rural and urban locations in 2010 were:

Upland site: At HMP Dartmoor prison farm, *Q. robur* buds had formed but not bursting on 20th April 2010 (early phase 2), early stages of bud-burst (early phase 3) on most *Q. robur* noted on

28th April 2010. Urban sites: At Digginit bud-burst was occurring in most saplings with many new leaves (phase 3). At Laira bud-burst was occurring with some new leaves (phase 2 and early signs of 3) . Rural sites: At Broadley bud-burst was occurring with one or two new leaves (phase 2 and early 3) At Dartington bud-burst with many new leaves (later stages of phase 3). Control: First signs of bud-burst recorded on 10th April (phase 3) with all new leaves by 20th April although many red (late stage phase 3 early stage phase 4).

Season and Environment.

Seasonal F_v/F_m was around 0.75-0.78 in rural and urban sites, increasing to 0.82 by July 2010 and remained high throughout the year in *Q. ilex*. However F_v/F_m experienced a drop in January 2011 but had already recovered by April 2011 (Figure 4.10a on page 64). The upland site followed a similar pattern with a more pronounced dip during the colder months (dropping to around 0.5 in November and January). mean *PI* values followed similar patterns to mean F_v/F_m although more variability was experienced (Figure 4.10b on page 64) , in particular, the combined urban sites show much higher PI values than the rural sites (Figure 4.10b) and reached maximum values around 12 for *Q. robur* and 14 for *Q. ilex*. For seasonal A_n in *Q. robur* all combined site characters began low at around 4 $\mu mol\ m^{-2}\ s^{-1}$ in May 2010 and increased to a maximum in July 2010, although this maximum was lower at the upland site (8.5 $\mu mol\ m^{-2}\ s^{-1}$) and as high as 16 $\mu mol\ m^{-2}\ s^{-1}$ for the rural sites (Figure 4.9a on page 63). The same pattern occurred during the next season although A_n was lower in general. This was especially so for the urban sites which had a combined mean of around 6 $\mu mol\ m^{-2}\ s^{-1}$ in July 2011, compared with 12 $\mu mol\ m^{-2}\ s^{-1}$ for the rural sites (Figure 4.9a A on page 63 . *Q. ilex* had a lower A_n in general (around 8 $\mu mol\ m^{-2}\ s^{-1}$) and all sites achieved this maximum in July 2010 although for the upland site, a later bud-burst meant Spring A_n was low (Figure 4.9a B on page 63). *Q. ilex* maintained maximum rates throughout the winter months in combined rural and urban sites although the rural sites increased A_n to 10 $\mu mol\ m^{-2}\ s^{-1}$ in July 2011 while the urban site reduced mean A_n from March to July 2011 (8 - 6 $\mu mol\ m^{-2}\ s^{-1}$). In contrast the upland site reduced A_n in march 2011 increasing steadily to another maximum in July 2011 of 8 $\mu mol\ m^{-2}\ s^{-1}$ (Figure 4.9a B).

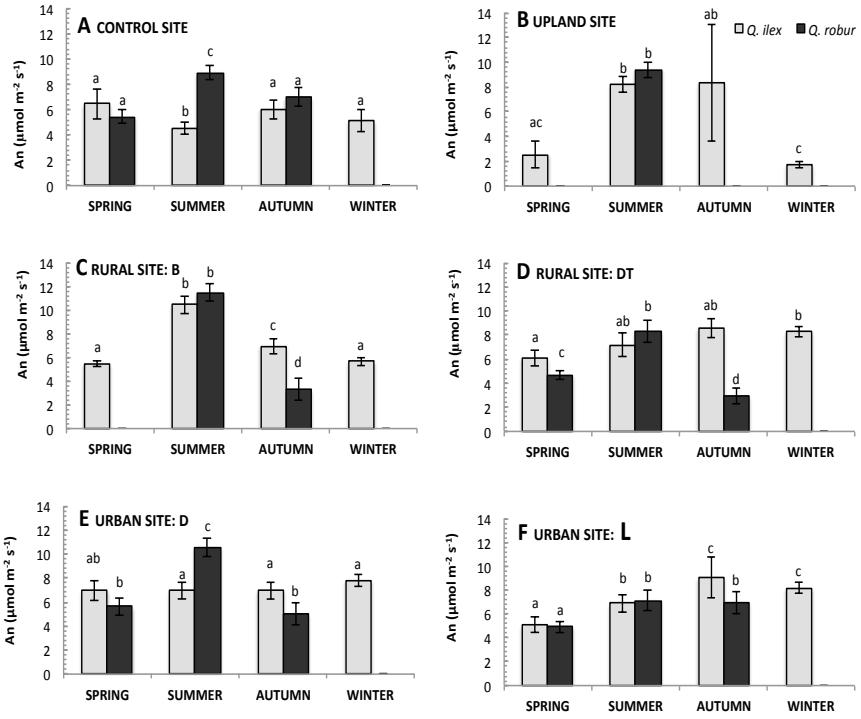
At the upland site, *Q. ilex* was unable to maintain high assimilation rates in winter (as low as 2 $\mu mol\ m^{-2}\ s^{-1}$) and this corresponded with lowered *Gs*, and photosynthetic efficiency for this site (figures 4.9aA, 4.9bA, 4.10aA, 4.10bA on pages 63, 63, 64, 64). However, *Q. ilex* was able to maintain higher A_n at the rural sites for all seasons although there was a more marked increase in summer at rural site B compared to the other seasons at this site (Figure 4.9aC). Photosynthetic efficiency was also compromised during the winter months in *Q. ilex* despite its efforts to maintain assimilation rates (Figure 4.10a, 4.10b). Although both rural sites are a similar distance from Dartmoor the rural site B is located on higher ground (80masl) and experienced lower winter temperatures and rainfall than the other rural site DT (see section 3 Figure 3.4 on page 39). The urban site show *Q. ilex* A_n was uniform across the seasons except at L summer assimilation rates were lowered (Figure 4.9aE). This corresponded with reduced *Gs* at this site and although photosynthetic efficiency values were lowered during winter for the urban sites, L experienced lowered PI across all seasons (Figure 4.10bE, page 64).

In comparison, although *Q. robur* A_n is generally lowered during spring/autumn, this species enjoyed high A_n at most sites during the summer and only showed lowered A_n at the urban site L. Despite these high summer assimilation rates at the upland site, photosynthetic efficiency was also low at the upland site in *Q. robur*.

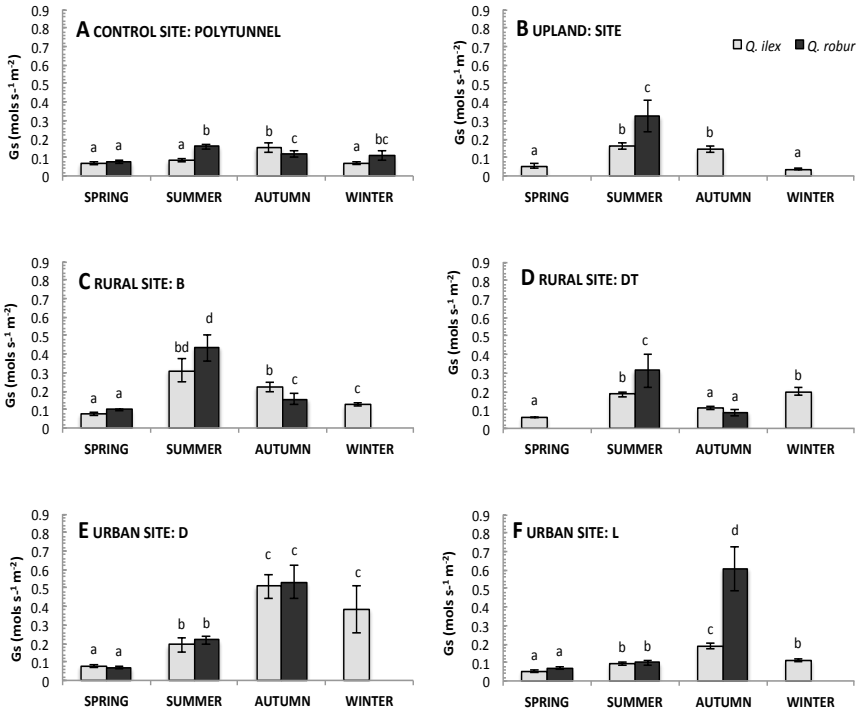
4.5.6 Results: Sun, Shade and Environment.

Generally upper leaves had higher mean A_n than lower leaves, although *Q. ilex* showed more distinction between upper and lower leaves than *Q. robur*. In fact those sites where *Q. robur* had reduced productivity, the subsequent lack in distinction between upper and lower leaves was due to less foliage for this species. This was most evident at rural site B, urban site L and the upland site (figures 4.11 and 4.12).

Mean *Gs* at the rural sites followed a similar pattern to A_n and was higher in upper than lower leaves in *Q. ilex* whereas for *Q. robur* there was no difference. At the upland site both species had low *Gs* and there was no differences between leaf position and although lower at the urban sites there was no distinction between upper and lower leaves in *Q. ilex* increased *Gs* in *Q. robur* at D was greater in upper than lower leaves (Figure 4.11).

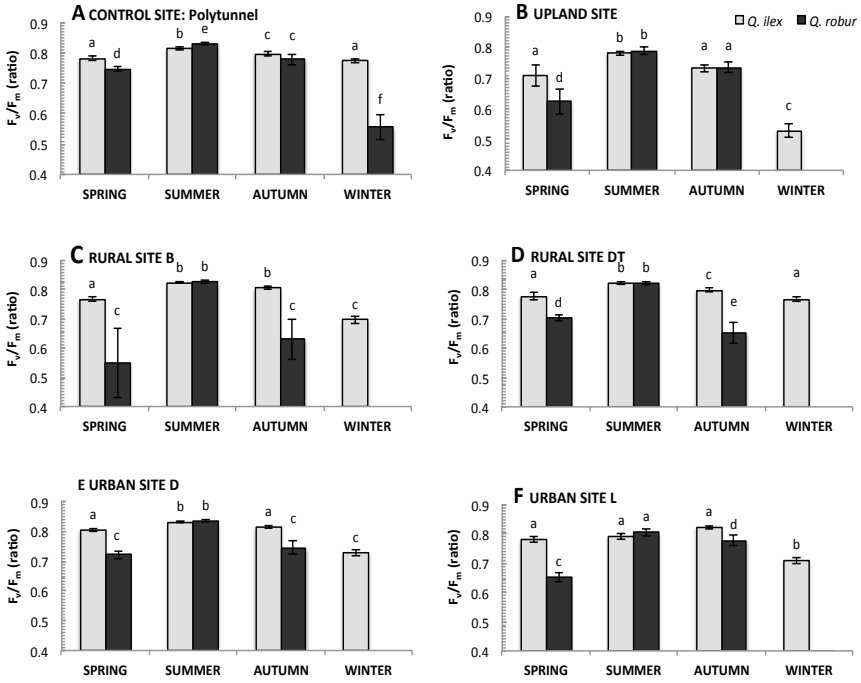


(a) Mean Seasonal Assimilation rates A_n.

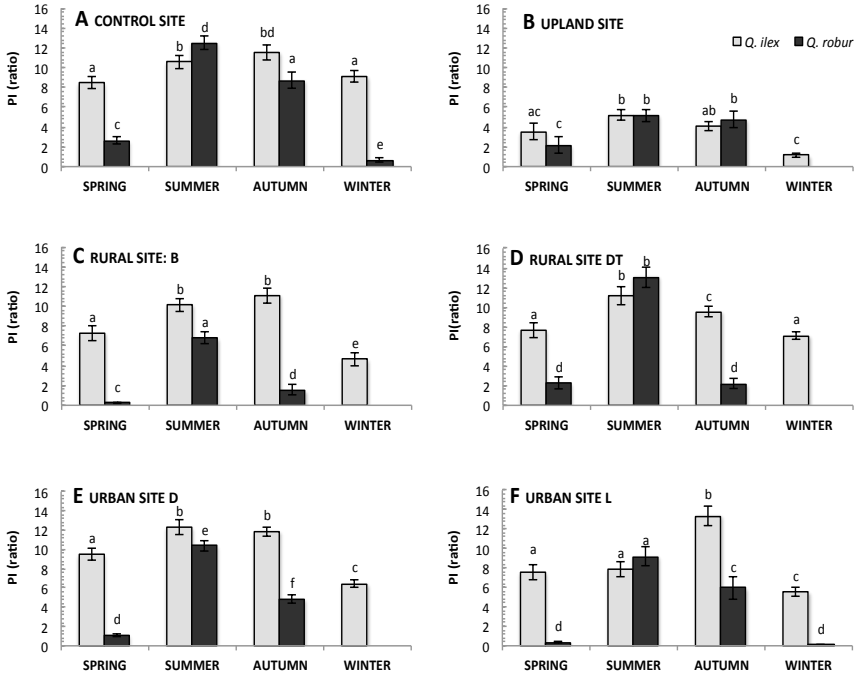


(b) Mean Seasonal Stomatal Conductance (Gs).

Figure 4.9: Species differences in a) net assimilation rate A_n and b) stomatal conductance (*Gs*) for season and sites (means $\pm$95%*CI*). Significant differences between species and seasons at each site is shown by different letters ($p<0.05$).



(a) Mean seasonal variable fluorescence over maximum fluorescence ratio F_v/F_m for each site.



(b) Mean seasonal performance index *PI*for each site

Figure 4.10: Species and seasonal differences in a) variable fluorescence over maximum fluorescence ratio F_v/F_m and b) performance index *PI* for each site (means $\pm 95\%CI$). Significant differences between species and seasons at each site is shown by different letters ($p<0.05$).

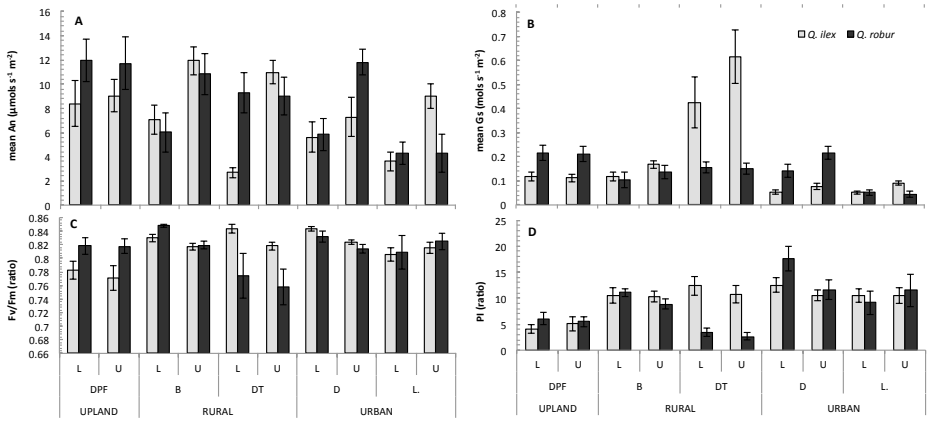


Figure 4.11: Species and environment differences in Upper (U) and Lower (L) canopy leaves. Individual sites used (Urban sites L and D, rural sites DT and B and upland site DPF) for, A) net assimilation rate A_n, B) stomatal conductance *Gs*, C) Maximum yield of primary photochemistry in photosystem II shown by the variable fluorescence, maximum fluorescence ratio F_v/F_m, and D) chlorophyll fluorescence performance index *PI* (means $\pm 95\%CI$).

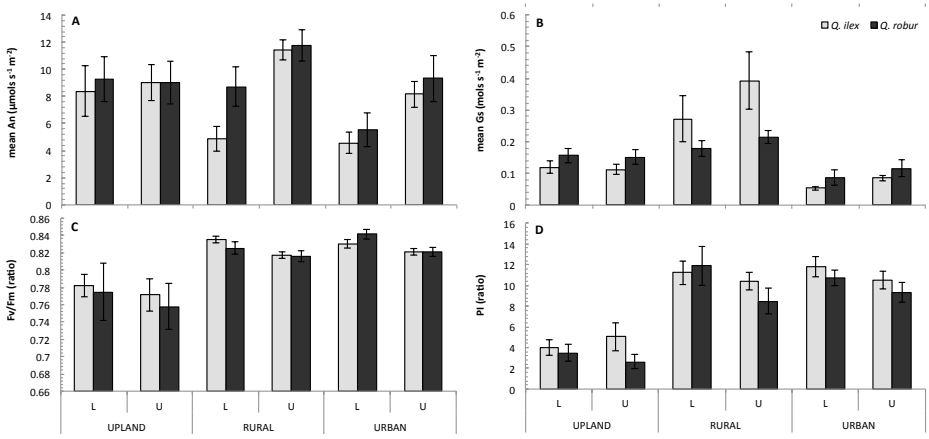


Figure 4.12: Species and environment differences in Upper (U) and Lower (L) canopy leaves. Sites are pooled into urban, rural and upland for, A) net assimilation rate A_n, B) stomatal conductance *Gs*, C) Maximum yield of primary photochemistry in photosystem II shown by the variable fluorescence, maximum fluorescence ratio F_v/F_m, and D) chlorophyll fluorescence performance index *PI* (means $\pm 95\%CI$).

In both rural and urban sites, F_v/F_m and *PI* were reduced in upper leaves compared to lower for both species (Figure 4.12). At the upland site, F_v/F_m and *PI* were generally low for both species. Mean values were generally higher in *Q. ilex* but there was no distinction between upper and lower leaves for either species at this site (see table 4.3, page 66 for ANOVA table).

Table 4.3: ANOVA table: Effects on physiological variables of leaf position (upper U or lower L) including site and species interactions for field grown saplings showing F statistics and p<0.05 '*', p<0.01 '**' and p<0.001 '***' and p<0.05 not significant 'ns'.

Analysis of Variance Table

Physiological variables		A_n		Gs		$iWUE$		F_v/F_m		PI	
FACTORS	Df	F	p<	F	p<	F	p<	F	p<	F	p<
SPECIES	1	13.2	***	46.2	***	95.3	***	4.5	*	255.8	***
SITE	4	7.4	***	28.8	***	44.8	***	125.7	***	114.0	***
LEAF POSITION	1	80.8	***			8.4	**	159.0	***	105.2	***
SPECIES:SITE	4	66.6	***	15.8	***	3.6	**	57.3	***	40.5	***
SPECIES:LEAF POSITION	1	0.7	ns			4.1	*	4.6	*	4.8	*
SITE:LEAF POSITION	4	3.1	*			1.3	ns	2.8	*	1.5	ns
SPECIES:SITE:LEAF POSITION	4	5.9	***			2.7	*	4.1	**	4.1	**
Residuals	3195							5281			

4.6 Discussion.

Differences in assimilation rates and photosynthetic efficiency indicate *Q. roburs* preference for fertile soils (Boisvenue and Running 2006) as assimilation rates and efficiency tended to be higher in potted plants. In contrast, *Q. ilex* thrives on poorer, drier soils (Barbero et al. 1992). Dark respiration was lower in *Q. ilex* than *Q. robur* and tends to be lower in Mediterranean evergreens compared to deciduous species in general (Gratani et al. 2008), however although extensive experiments were not undertaken to deduce changes in respiration with temperature recent experimental evidence suggests that although respiration initially increases with increasing temperatures, acclimation occurs and respiration returns to pre temperature increase values (VanOijen et al. 2010). Additionally, daytime respiration is thought to be similar to that of night time respiration as any extra CO_2 used in respiration is taken back into the photosynthetic process (Bolstad et al. 2003).

Both oaks studied here are late successional species, however my research indicates that *Q. robur* is more shade tolerant than the Mediterranean *Q. ilex* , deciduous oak saplings have been shown to be less tolerant to high light than *Q. ilex*, in a Mediterranean environment where de-

ciduous oaks have to establish under thick shade (Gomez-Aparicio et al. 2006). Additionally, during April when temperatures were mild *Q. ilex* made the most of light levels to increase assimilation rates at higher light levels than it did during the summer. This strategy enables it to make the most of winter light levels, which it can't do during the summer when drought and high temperatures are more likely to occur. However. this increase in light saturation level during early spring led to a reduction in photosynthetic efficiency during the winter months and leaves showed signs of reversible photo-inhibition during this season. Another study monitoring photosynthetic traits in *Q. ilex*, growing in two different climatic conditions in Italy, showed that light response increased with temperature, maximum assimilation rates were lower during winter (at light saturation) and photosynthetic efficiency was reduced, shown by F_v/F_m in leaves during winter at the colder site (Gratani, Pesoli, Crescente, Aichner and Larcher 2000). However earlier laboratory experiments proved that at optimum temperatures light saturation in *Q. ilex* was around 1000 $\mu mol\ s^{-2}$ (e.g. Larcher 1969) cited in (Gratani, Pesoli, Crescente, Aichner and Larcher 2000).

In this thesis *Q. ilex* saplings showed a much more conservative response to summer temperatures and light; closing stomata and down regulating photosynthesis regardless of water availability as protection to the photosynthetic apparatus occurs. This is a conservative water use strategy and has also been shown in the Mediterranean deciduous Turkey oak (Tognetti et al. 2007). However, the deciduous species does not down regulate photosynthesis in response to higher temperatures and therefore its drought response is slower than *Q. ilex*. The ability of *Q. robur* to increase assimilation in warmer weather leaves this species more vulnerable to drought. This strategy even competes within its own sympatric species, *Q. petrea*, which had 10-15% higher intrinsic water use efficiency than *Q. robur* when grown in full sun despite *Q. robur* net assimilation rates being higher (Ponton et al. 2002).

In order to reduce the trade off in assimilation rate during the summer *Q. ilex*, increases its photosynthetic light response during milder weather in the cooler months. This is evidence of its plasticity towards sun/shade adaption where carbon fixation is is favoured in high light environments and light harvesting in low (Friedrichs et al. 2009) (this is also shown in LMA

responses shown in chapter 5). However lower photosynthetic efficiency at lower temperatures makes this species more vulnerable to photoinhibition (Camarero et al. 2012).

The urban environment exerts stresses on oak growth through limited water availability as well as heat and pollution. Saplings at the urban sites exhibited different responses. It is hard to separate effects of pollution and water stress through field studies alone although further work on C_{leaf} and N_{leaf} showed higher levels of N_{leaf} in *Q. robur* at the urban versus rural environments, evidence that *Q. robur* accumulates *N* in leaves through *N* deposition in urban environments (Searle et al. 2012). This may mean that *Q. robur* is able to make use of *N*, and not be inhibited by it. In fact, one study has shown that *Q. robur* was more affected by water availability than particulate pollution in an urban environment (Ferretti et al. 2002), although another showed that *Q. robur* leaves were more susceptible to urban pollution than *Q. ilex* (Calatayud et al. 2011).

4.7 Conclusion.

My research showed that *Q. robur* had higher mean assimilation rates than *Q. ilex*. However, *Q. ilex* had higher assimilation rates at the urban site L where soils were drier and saplings were more exposed, despite photosynthetic efficiency being lower in *Q. ilex* at this site. Net carbon assimilation A_n and the more sensitive chlorophyll fluorescence parameter *PI* were significantly lower in *Q. robur* than *Q. ilex* at rural site B. This site was situated on the eastern Dartmoor 'fringe', having the second lowest air temperatures after the upland site and second driest site after the urban site L.

Higher $iWUE$, in both species, at the exposed urban site L indicated water stress at this site. Struve et al. (2009) found that *Q. robur* grew the tallest and used more water when compared with *Q. ilex* growing in an urban environment, indicating that the conservative water use of the evergreen *Q. ilex* would give this species an advantage in urban environments, while the colder, wetter upland environment should give the deciduous *Q. robur* the advantage as its deciduous habit gives it protection during winter cold stress.

Although photochemical efficiency is lower in young *Q. ilex* in response to cold stress. Tem-

peratures above 5 °C tend to enable increased assimilation in this species. *Q. ilex* has been shown to represent a complex species (Barbero et al. 1992) and phenotypic plasticity within this species enable it to withstand climatic extremes (Gimeno et al. 2009).

In conclusion, the response of each species was site specific with *Q. ilex* net assimilation and photosynthetic efficiency higher at those sites that were drier compared with *Q. robur* and colder average temperatures reducing photochemical efficiency in both species although *Q. ilex* was able to increase assimilation rates when mean temperature were above 5 °C.

Chapter 5

Leaf Traits.

Analyses of leaf traits can be a useful addition when measuring productivity and carbon sequestration potential in relation to environment. Specific leaf area (SLA), leaf nitrogen content (N_{leaf}) and leaf carbon (C_{leaf}) are closely coupled with net photosynthesis and relative growth rates, where the inverse of SLA, leaf mass per area (LMA), increases along with N_{leaf} and net assimilation rates and low SLA is generally combined with higher C_{leaf} concentrations and faster growing species. Leaf traits were measured in two year old saplings of *Q. robur* L. and *Q. ilex* subs. *ilex*.L growing at two urban , two rural and one upland site in and around Plymouth SW England. Leaf traits were analysed for differences between species and sites in relation to an urban, rural and upland site using mixed general linear models. SLA and N_{leaf} were higher in *Q. robur* than *Q. ilex* in common with generalised leaf traits for evergreen and deciduous broadleaved species. However, C_{leaf} and N_{leaf} were higher in *Q. ilex* sun versus shade leaves. Mean SLA tended to decrease with increasing light in *Q. robur* while SLA increased at sites where mean temperatures were lower and this effect was greater in potted saplings. Mean SLA was less variable in *Q. ilex* although a similar pattern was seen at the upland site. This chapter then discusses the relationship with SLA and environment. However, further evidence for the observed trends is linked to biomass allocation and general growth strategies employed by deciduous and evergreen species.

5.1 Introduction.

Commonly, leaf traits are used to measure growth and productivity in trees. Leaves as the main site of carbon fixation and used alongside more direct growth and biomass experiments, can give realistic estimates of carbon sequestration potential (e.g., Cornelissen et al. 2003). A leaf trait of particular importance, is specific leaf area SLA (leaf area / leaf weight, $cm^2\ g^{-1}$), or

its reciprocal *LMA* a much used parameter that is related directly to relative growth rate (RGR) through assimilation rates (A_n) and leaf area ratio (LAR)[1]. Other commonly used leaf traits are leaf carbon and nitrogen concentrations which can be used to infer construction costs in relation to leaf habit and environment. It follows that leaf traits are known to change with environmental pressures such as drought (Ogaya and Penuelas 2006), pollution and average temperatures (Cornelissen et al. 2003) and are considered as both morphological and physiological adaptations to their environment (e.g. Gratani and Bonmbelli 2001). As such leaf traits can aid interpretation when comparing functional differences in evergreen and deciduous trees.

5.1.1 Specific Leaf Area.

Generally *SLA* increases with decreasing irradiance, and leaves developed in high light tend to be smaller and thicker with correspondingly low *SLA* (Evans and Poorter 2001). This is due to an increase in palisade cells and photosynthetic enzymes that are necessary to maximise the carboxylation process (Lichtenthaler et al. 2007). On the other hand, shade adapted leaves are larger and thinner and contain more light harvesting pigments, arranged to avoid self shading, and therefore increasing the area to mass relationship of the leaf (Lichtenthaler et al. 2007).

5.1.2 Leaf Nitrogen.

Leaf nitrogen (N_{leaf}) tends to be associated with concentrations of the enzyme, Ribulose-1 5-Biphosphate Carboxylase Oxygenase (RuBisCO) and thus increased *N* leads to greater carbon fixation in leaves (Grime 2002; Koike et al. 2004; Lorenz and Lal 2010). However, light, temperature and drought modify the effect of *N* on photosynthesis (Lorenz and Lal 2010), in particular N_{leaf} tends to increase through the canopy along with increasing light availability (Niinemets and Tenhunen 1997).

The foliar deposition of *N* is an important issue (Kennedy 2003) and has been shown to increase assimilation at low concentrations while having a detrimental effect at higher concentrations (WHO 2011). Despite this, *N* deposition in the UK does not seem to affect oaks (Kennedy 2003).

[1] leaf weight ratio *LWR X SLA*, where *LWR* is the ratio of leaf weight to plant weight see equation 6.7 (Fitter and Hay 2002)

A substantial amount of N_{leaf} is utilised by photosynthetic organs within the leaf, and thus increasing N_{leaf} is commonly associated with increasing carbon assimilation rate (Takashima et al. 2004). Leaf level net assimilation and leaf nitrogen ratio (A_n/ N_{leaf}) is known as photosynthetic nitrogen use efficiency ($PNUE$) as 50% of N_{leaf} generally being used for photosynthesis (Takashima et al. 2004). Evergreens generally have lower $PNUE$, as do species growing at higher altitudes where an increase in nitrogen associated with cell wall formation may occur, or, in other areas where heat and drought stress induce stomatal control of photosynthesis and leaf cuticles are thickened to reduce water loss (Takashima et al. 2004).

5.1.3 Leaf Traits and Environment.

Changes in leaf traits such as *LMA* are an indication of adaptation to stress, and species adapted to drier environments such as *Q. ilex* are known as 'schlerophylls'. The degree of adaptation to xeric environments is measured on an index of 'schlerophlly' which starts at $LMA > 7.5\ mg\ cm^{-2}$ (Filippo 2008).

Q. ilex has an average leaf life span (LL) of 36 months (Gratani and Bonmbelli 2001) while *Q. robur* LL can average from 160 to over 200 days (this research), depending on site and environmental conditions (M. et al. 2006; Bobinac et al. 2012). Both species are episodic (Kozlowski 1971) and produce a second flush in August. *Q. ilex* can continue to produce new leaves periodically throughout the year if conditions are mild (personal observation). *Q. robur* retains leaves until late October (again depending on environment) although they can remain on a young tree much longer despite being photosynthetically inactive (marcescence[2]) (Morin et al. 2010).

In my research, *Q. ilex* saplings first year leaves were still important contributors to photosynthetic gain as second year leaves but as third year leaves their photosynthetic rates were halved. For this reason mature current year leaves (CYL) were used for analyses of leaf traits although some data on developing current year leaves is presented separately.

1. General working hypotheses for Leaf traits are:

    1.1. There is a significant difference in leaf traits between species and leaf position (up-

[2]Marcescence: The retention of dead plant anatomy, here referring to leaves.

per and lower).

1.2. There are significant differences in leaf traits between species and sites (with interactions).

1.3. Are there any significant effects of site character (SC: URBAN RURAL or UPLAND) on leaf traits and is this effect different for upper and lower leaves.

5.2 Methodology.

Mature leaves from the previous years growth were still important contributors to photosynthetic gain and assimilation rates were only reduced in three year old leaves, mainly through self shading of foliage (data not shown). With this in mind mature current year leaves (CYL) were used for analyses of leaf traits although, for *Q. ilex*, as the year progressed the sampled leaves became previous years foliage as second flush leaves matured. In some cases where saplings were under stress and leaves matured and died quickly, some data on developing current year leaves is also presented as new leaves (NL).

For each site and condition two leaves were taken from 10 saplings: One leaf from upper and one from lower canopy were measured in order to see if there were differences in 'sun' and 'shade' leaves. Where there was no distinction between upper and lower foliage it was expected for there to be no difference in results due to lack of self shading and therefore 'shade' leaves not present.

Fresh leaves were sealed in bags and transported straight to the laboratory in a cold box for immediate measurement of fresh weight. After weighing, leaf area (LA) was determined using the LiCor Leaf area Analyser and each leaf dried in the oven at 70°C until dry (two consecutive weights the same in a sub sample of each set). Dry weights were then recorded and *SLA* and dry matter content (*LDMC*) determined (*LDMC* data not shown). Mass based leaf carbon C_{leaf} and N_{leaf} were analysed as percentage concentrate in 2 mg of leaf , using dried and ground leaf samples from each treatment level (see chapter 2 section 2.3.3 for complete methodology). Leaf traits quantified are; *SLA* (sometimes discussed as *LMA* within the wider literature), mass based N_{leaf} and C_{leaf}. N_{leaf} was determined on a mass basis and N_{leaf} on an area basis was

determined by multiplying the mass based N_{leaf} by *LMA* (Wright et al. 2005).

5.2.1 Statistical Analyses.

2010 leaf traits were analysed using a mixed general linear model (MGLM) after making sure the data met the assumptions for ANOVA (Levenes test, p>0.05). All mixed linear models were performed using the lm(model) command in R version 2.10 (R 2009). To avoid over complication of interactions first the model was run using ENVIRONMENT and its interaction with SPECIES (SP) to look for species differences within the urban, rural and upland environments; a second model was produced using SITE (S) nested in ENVIRONMENT and analysed with planting TREATMENT (field grown (G) or potted (P)) and LEAF POSITION (LP) plus interactions. Interval plots show means $\pm$ 95% confidence intervals and descriptive statistics within text give means, standard deviation (StD) minimum, maximum, skew and sample number (N) (Table 5.1). Table 5.1 also gives tukeys honest significance test (HSD) results for differences between sites for each species.

5.3 Results.

SLA and N_{leaf} were significantly higher in *Q. robur* than *Q. ilex*, (*SLA*, 16.9 $\pm$ 6.6: 7.6 $\pm$2. (cm^2 g^{-1}), N=539 and N_{leaf} 2.4 $\pm$ 0.32: 1.5 $\pm$ 0.23 (%), N=60). C_{leaf} was generally around 47 % in both species, however there were also significant effects of site and species site interactions where for *Q. ilex* rural site DT was significantly higher C_{leaf} and in *Q. robur* urban site D had signicantly lower C_{leaf} (48.4 $\pm$ 0.95: 46 $\pm$ 1.35 (%), N=12 respectively)(see figures 5.4 to 5.7, pages 82 to 85). To summarise *Q. robur SLA* was highest at upland DPF (150.4 $\pm$ 54.43) and lowest at L (124.2 $\pm$ 21.43). *Q. ilex SLA* was also lowest at urban L (59.06 $\pm$ 12.4) but highest mean values were found at B for this species (76.27 $\pm$ 32.08) (see Table 5.1).

5.3.1 Effect of the Urban, Rural, Upland Environmental Gradient.

Leaf traits were affected by ENVIRONMENT, however, the upland environment has a greater affect on *SLA* and N_{leaf}, while differences in environment in C_{leaf} are also site specific (see figures 5.1, 5.2 and 5.3. In *Q. robur* the higher *SLA* at the upland site (Figure 5.1) accounts for the difference in *SLA* with SC while for *Q. robur* C_{leaf} was lower than in *Q. ilex* although at the

upland site *Q. robur* had greater C_{leaf} than rural and urban sites. The higher C_{leaf} at rural DT and lower at urban D account for the significant effects of ENVIRONMENT on *Q. ilex* C_{leaf} (Figure 5.2). N_{leaf} is also site specific and although higher in *Q. robur* the difference between sites mean there is little difference between the rural and urban environments and again it is the upland site that gives the significant effect of this factor (see Table 5.1 for descriptive statistics and Table 5.2 for model results).

5.3.2 Effect of Condition: Differences between Potted and Field Grown Saplings.

Although *Q. robur SLA* was higher in DPF potted saplings (Figure 5.7), there was no significant effect of TREATMENT or interactions with TREATMENT and other factors on C_{leaf} or *SLA* (ANOVA results not shown). However, N_{leaf} was significantly affected by TREATMENT and this interacted with all other factors in the model (Table 5.3). N_{leaf} was significantly lower in field grown saplings G at the upland site DPF for both species (Figure 5.6 a and b) although this effect was stronger for *Q. robur*. Pots were less variable for *Q. robur* N_{leaf} although the rural site B was significantly lower than urban D, and rural DT (Figure 5.6).

5.3.3 Sun and Shade Effects.

No interactions with TREATMENT were observed therefore a simplified model was produced using full interaction plot with SP*S*LP (Levenes test, P>0.05 for all leaf traits) (table 5.4).

SLA.

The effect of LP interacted with SP and S for *SLA* (Table 5.4 and Figure 5.7). In *Q. robur* mean *SLA* was significantly lower in upper leaves and interacted with SITE to show more constrained and lower *SLA* at rural DT and urban L especially in upper leaves (Figure 5.7), however, at the upland site, DPF, there is no difference between upper and lower leaves and indeed there was no distinction between lower and upper canopy at this site in *Q. robur* and therefore no 'shade' leaves. *SLA* was less variable in *Q. ilex* although higher in both upper and lower leaves at rural B, there was no difference between upper and lower at this site. There were however, significant differences between upper and lower leaves at urban D and rural DT for this species an indication of the dense foliage see at this site giving a clear distinction between sun and

Table 5.1: Leaf traits: Specific leaf area SLA ($cm^2\ g^{-1}$), carbon and nitrogen % concentrations in 2mg leaf. Capital letters denote significant differences between sites for each species using Tukey's HSD which looks for differences in all pairwise means

Descriptive statistics for leaf traits

Variable		SITE	N	Mean	StDev	Minimum	Maximum	Skewness	Tukey
					Q. ilex				
SLA	Rural	B	38	71.4	33.3	29.6	161	1.56	A
		DT	40	60.8	15.12	39.45	97.6	0.74	AB
	Urban	D	39	56.8	10.5	39.17	82.3	0.56	B
		L	38	58.0	10.25	34.76	86.7	0.37	B
	Upland	DPF	17	63.3	11.81	47.93	86.6	0.32	AB
		C	18	52.6	9.64	35.34	69.0	0.19	B
N_{leaf}	Rural	B	12	1.35	0.155	1.07	1.55	-0.42	C
		DT	12	1.63	0.179	1.35	1.93	0	AB
	Urban	D	12	1.48	0.137	1.28	1.66	-0.19	BC
		L	12	1.76	0.208	1.35	2.10	-0.39	A
	Upland	DPF	12	1.39	0.193	1.17	1.71	0.74	C
	Control Site	CS	6	1.53	0.126	1.30	1.67	-1.39	ABC
C_{leaf}	Rural	B	12	47.5	0.687	46.5	48.5	0.12	AB
		DT	12	48.4	0.948	46.6	49.9	-0.17	A
	Urban	D	12	47.3	1.21	44.2	48.4	-1.61	B
		L	12	48.0	0.605	46.8	48.9	-0.68	AB
	Upland	DPF	12	47.8	0.896	46.7	49.6	0.53	AB
	Control Site	CS	6	46.9	0.61	46.4	48.0	1.26	B
					Q. robur				
SLA	Rural	B	40	160.6	42.7	96.8	295	1.22	B
		DT	39	138	29.4	72.6	220	0.24	B
	Urban	D	36	153	51.2	53.2	265	0.43	B
		L	32	145	27.1	101	200	0.57	B
	Upland	DPF	38	190	53.2	54.3	282	-0.4	A
	Control site	CS	20	154	37.7	81.3	245	0.52	B
N_{leaf}	Rural	B	12	2.21	0.231	1.83	2.70	0.47	C
		DT	12	2.68	0.16	2.32	2.83	-1.44	A
	Urban	D	12	2.55	0.196	2.23	2.87	0.07	AB
		L	11	2.51	0.19	2.25	2.79	0.09	AB
	Upland	DPF	12	2.09	0.362	1.635	2.57	0.13	C
	Control Site	CS	6	2.26	0.26	1.97	2.60	0.02	BC
C_{leaf}	Rural	B	12	47.2	0.69	46.3	48.9	0.9	A
		DT	12	47.7	1.18	45.7	49.3	-0.04	A
	Urban	D	12	46.5	1.36	44.7	49.2	0.41	AB
		L	11	47.7	1.15	46.2	50.7	1.94	A
	Upland	DPF	12	47.6	1.20	46.3	49.8	0.92	A
	Control Site	CS	6	45.5	0.274	45.0	45.7	-1.47	B

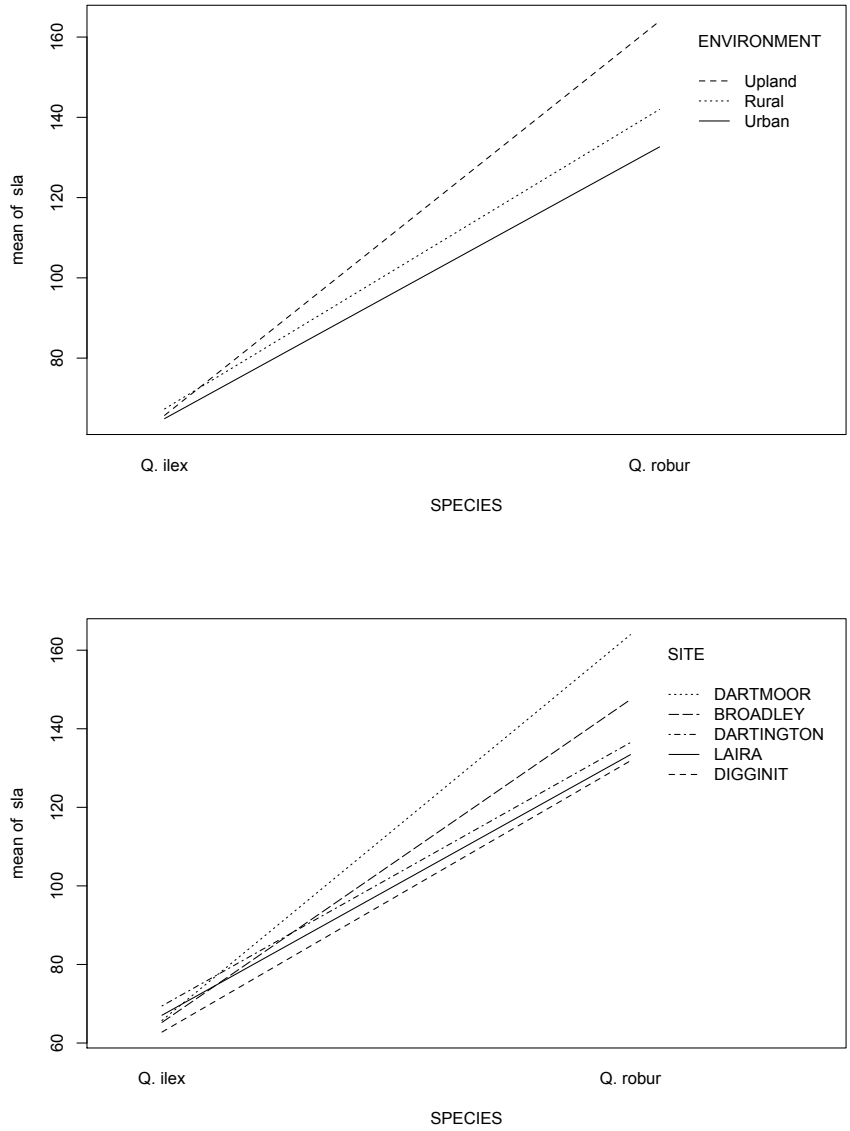


Figure 5.1: Interaction between SPECIES and both SITE (DARTMOOR is UPLAND(DPF), BROADLEY is rural, DARTINGTON is rural, DIGGINIT is urban and LAIRA is urban) and ENVIRONMENT (upland rural, urban). Means analysed with mixed linear models to highlight the site specific nature of environmental effects on leaf trait, *SLA* ($cm^2 g^{-1}$).

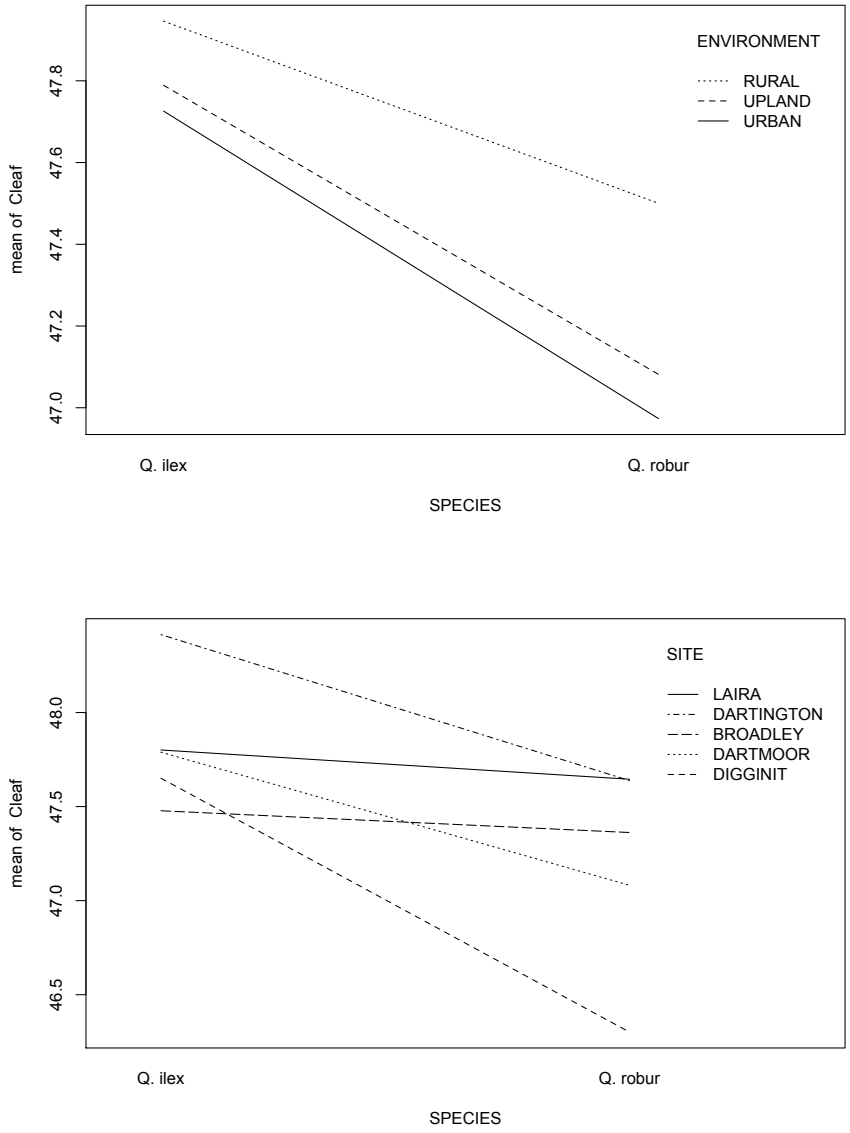


Figure 5.2: Interaction between SPECIES and both SITE (DARTMOOR is UPLAND(DPF), BROADLEY is rural, DARTINGTON is rural, DIGGINIT is urban and LAIRA is urban) and ENVIRONMENT (upland rural, urban). Means analysed with mixed linear models to highlight the site specific nature of environmental effects on leaf trait, CResult= C_{leaf} (mean % concentration).

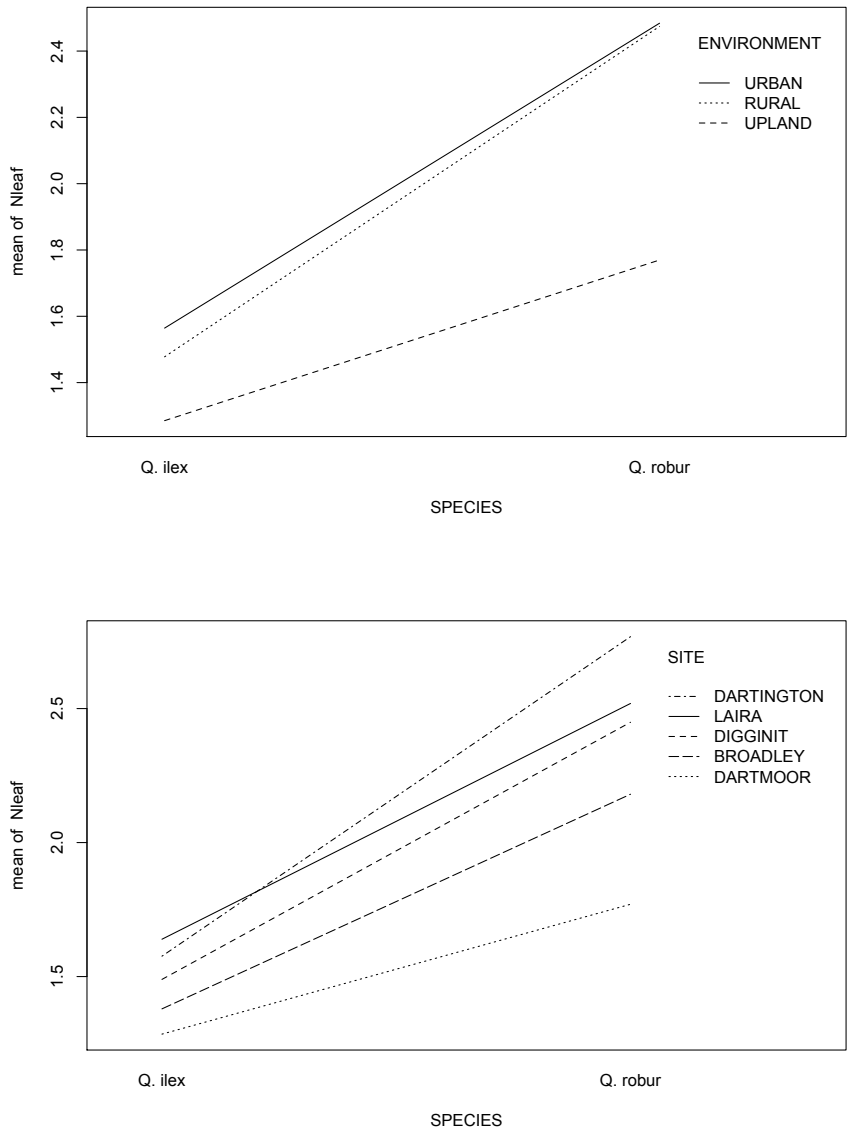


Figure 5.3: Interaction between SPECIES and both SITE (DARTMOOR is UPLAND(DPF), BROADLEY is rural, DARTINGTON is rural, DIGGINIT is urban and LAIRA is urban) and ENVIRONMENT (upland rural, urban). Means analysed with mixed linear models to highlight the site specific nature of environmental effects on leaf trait, NResult= N_{leaf} (mean % concentration).

Table 5.2: Species and environment (Urban, Rural and Upland) effects and interactions on leaf traits.

ANOVA table: Leaf Traits

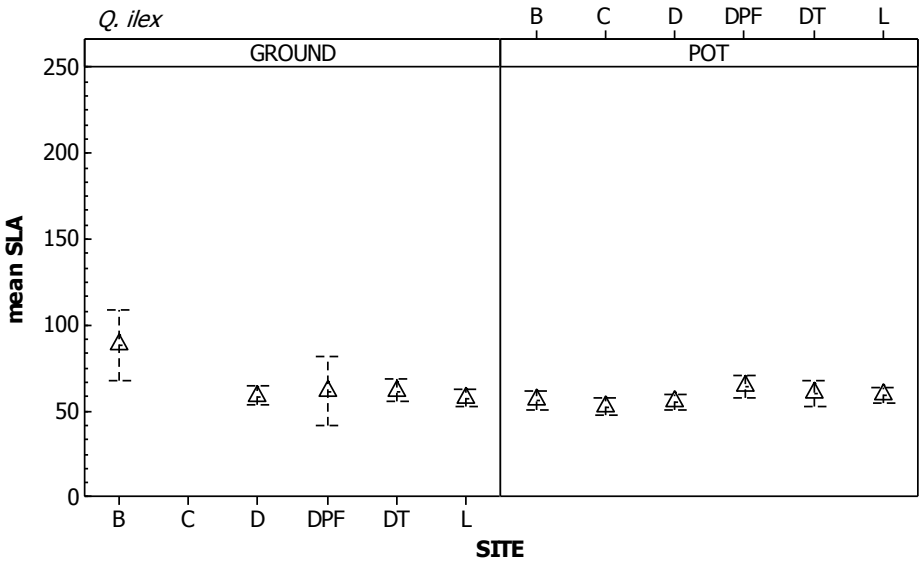
Response:		*SLA*		N_{leaf}		C_{leaf}	
	Df	F	sig.	F	sig.	F	sig.
SPECIES	1	411	***	408	***	9.38	**
ENVIRONMENT	3	12	***	10.1	***	7.6	***
SPECIES:ENVIRONMENT	3	6.86	***	1.82	ns	0.962	ns
Residuals SLA	338						
Residuals CN_{leaf}[1]	138						

[1] CN_{leaf} = C_{leaf} and N_{leaf} residual error

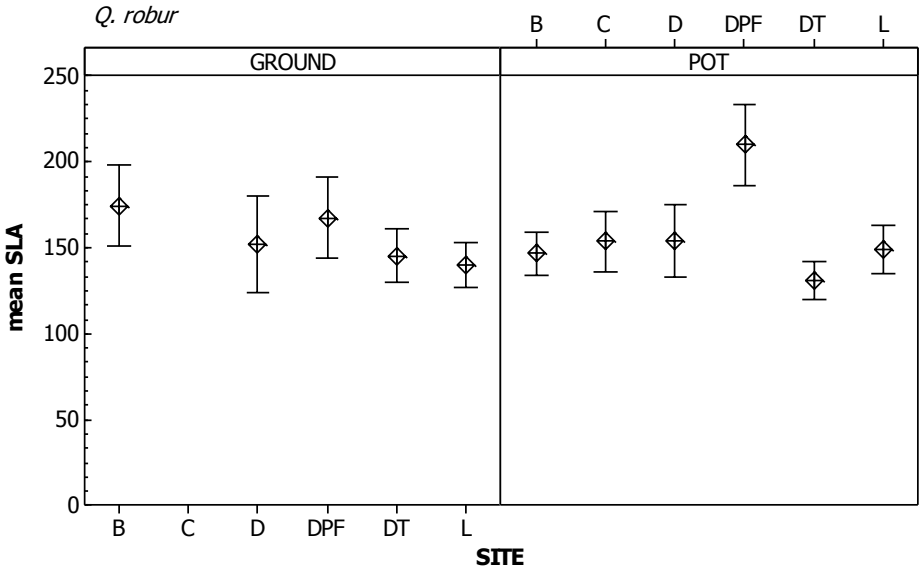
Table 5.3: Effect of soil treatment (POTS vs GROUND) on leaf traits (Species = SP, soil treatment = TREATMENT, Site = S (S nested within ENVIRONMENT). Significant levels, $p < 0.001 = ***$, $p < 0.01 = **$, $p < 0.05 = *$, p $\geq$ 0.05 = not significant (ns) (C_{leaf} ns, data not shown).

ANOVA table: Soil effects on leaf traits.

Response:	N_{leaf}		
	Df	F	Sig.
SP	1	794	***
S	5	23.8	***
TREATMENT	1	14.3	***
SP:S	5	4.65	***
S:TREATMENT	4	6.24	***
SP:S:TREATMENT	4	4.69	**
Residuals	109		

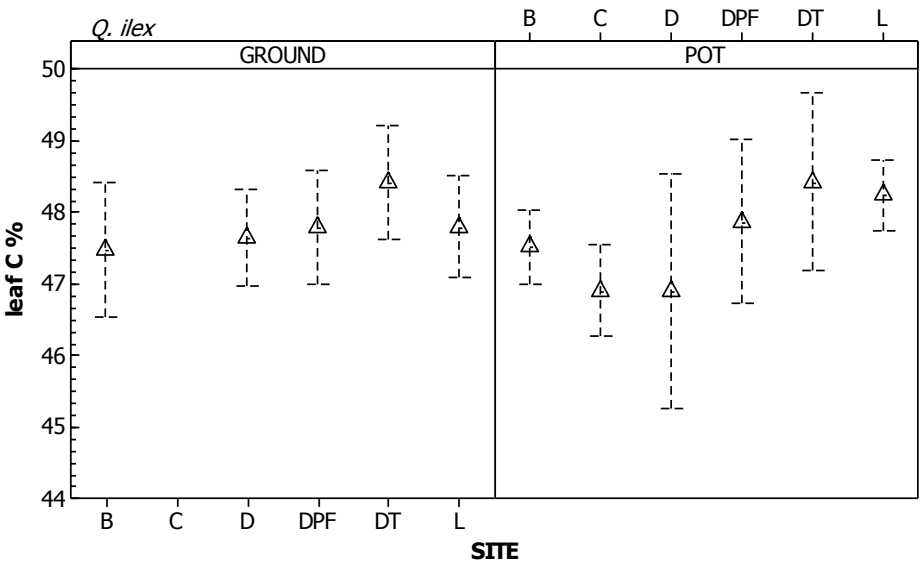


(a) Q. ilex

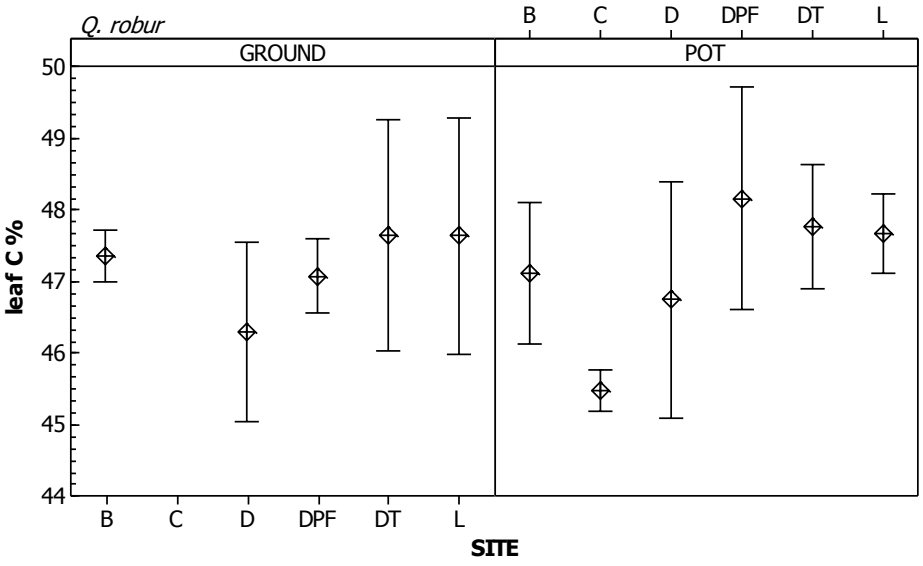


(b) Q. robur

Figure 5.4: SLA Species differences in SITE (Rural site BROADLEY (B),CONTROL SITE (C), urban site DIGGINIT (D), upland site DARTMOOR (DPF), rural site DARTINGTON (DT) and urban site LAIRA (L) and soil treatment (field grown (GROUND) and potted (POT)) plus interactions (means $\pm$95% CI).

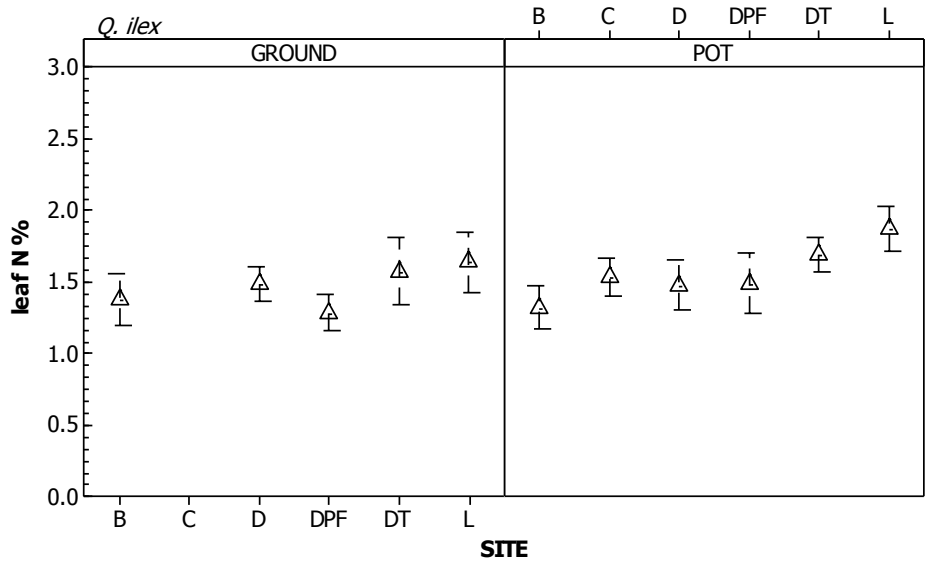


(a) Q. ilex

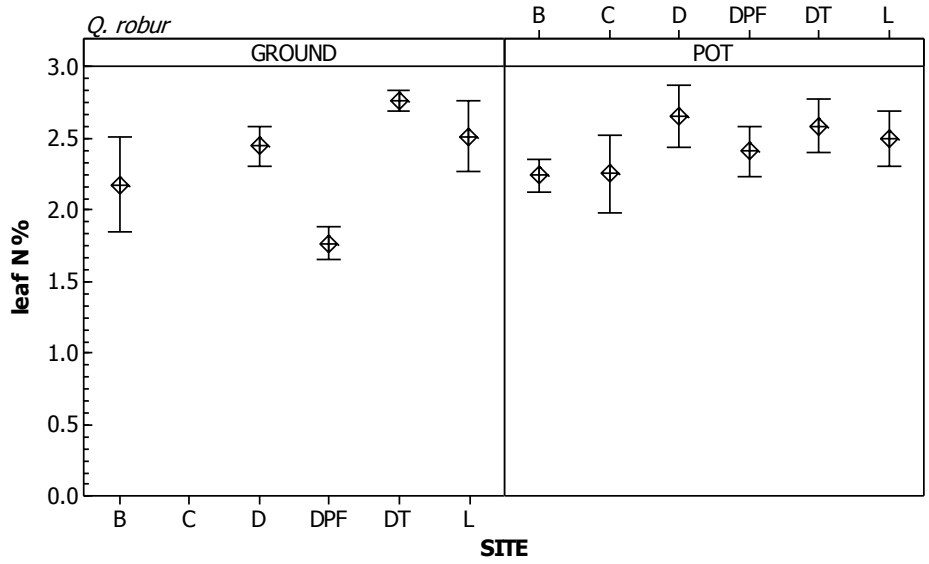


(b) Q. robur

Figure 5.5: C_{leaf}: Species differences in SITE (Rural site BROADLEY (B),CONTROL SITE (C), urban site DIGGINIT (D), upland site DARTMOOR (DPF), rural site DARTINGTON (DT) and urban site LAIRA (L) and soil treatment (field grown (GROUND) and potted (POT)) plus interactions (means $\pm$ 95% CI).



(a) Q. ilex



(b) Q. robur

Figure 5.6: N_{leaf}: Species differences in SITE (Rural site BROADLEY (B),CONTROL SITE (C), urban site DIGGINIT (D), upland site DARTMOOR (DPF), rural site DARTINGTON (DT) and urban site LAIRA (L) and soil treatment (field grown (GROUND) and potted (POT)) plus interactions (means $\pm$ 95% CI).

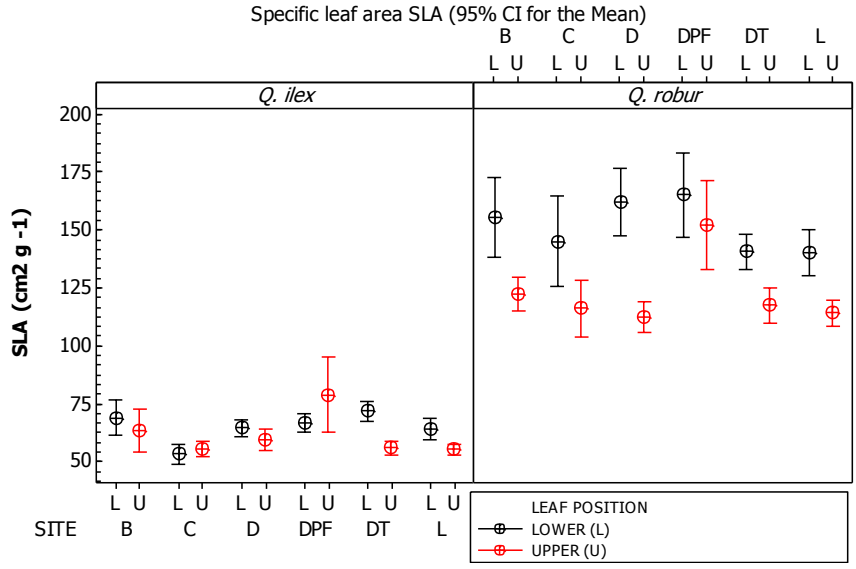


Figure 5.7: SLA ($cm^{-2}\ g^{-1}$): Species differences with S and LEAF POSITION (LP) interactions to quantify sun/shade effects (means $\pm$ 95% CI)

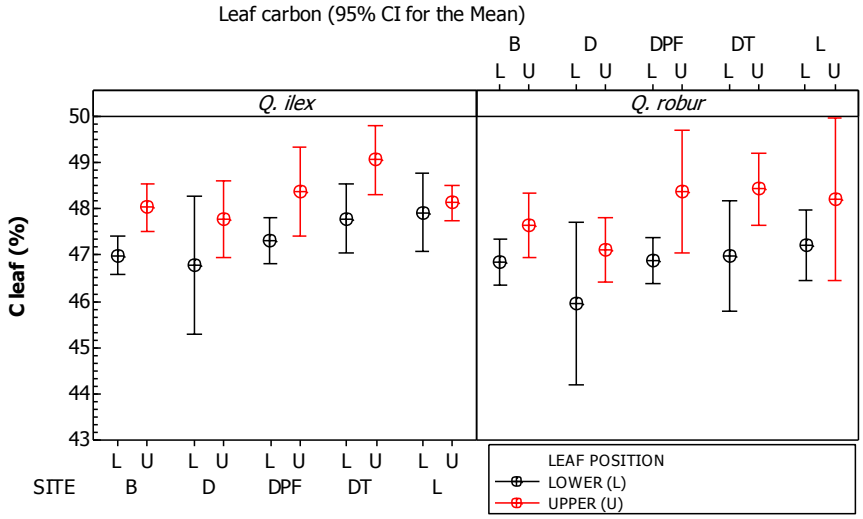


Figure 5.8: C_{leaf} (%): Species differences with SITE and LEAF POSITION (LP) interactions to quantify sun/shade effects (means $\pm$ 95% CI)

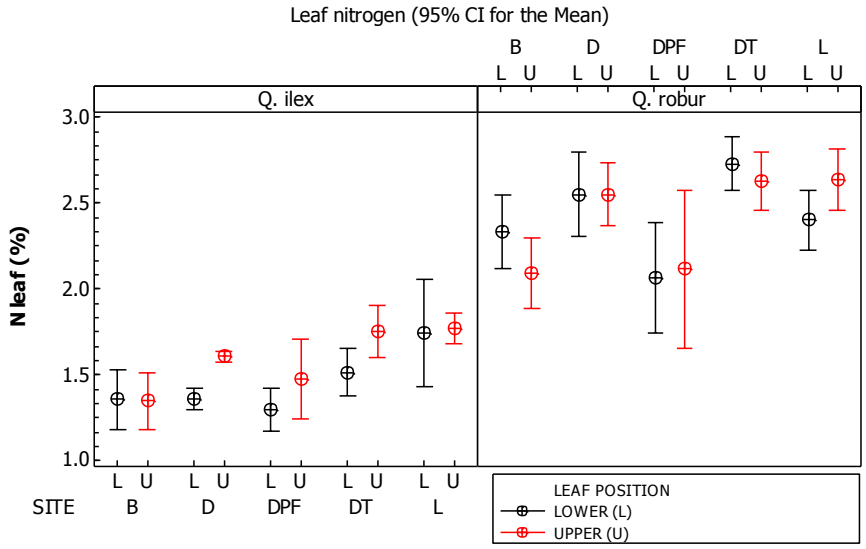


Figure 5.9: N_{leaf} (%): Species differences with SITE and LEAF POSITION (LP) (lower, upper) interactions to quantify sun/shade effects (means ±95% CI)

shade leaves (Figure 5.9).

C_{leaf}.

There were significant effects of each factor on C_{leaf}, however no interactions occurred (Table 5.4). C_{leaf} was higher in *Q. ilex* than *Q. robur* and higher in upper leaves than lower (Figure 5.8). In *Q.robur* upper leaves at CS had significantly lower C_{leaf} than upper leaves at the other sites (although there were no differences between upper and lower leaves at this site. In *Q. ilex* C_{leaf} was higher in upper leaves at DT although this was not significant (Figure 5.8).

N_{leaf}.

N_{leaf} was low in both species at the upland DPF in both upper and lower leaves (Figure 5.9). In *Q. robur* highest N_{leaf} was at rural DT in both upper and lower leaves and although N_{leaf} was reduced in lower leaves at L for this species upper leaves were comparable to DT values in *Q. robur* (2.6 ± 1.5 and 2.6 ± 1.6 (%)) (Figure 5.9). There were no significant differences between upper and lower leaves in *Q. robur* at any of the sites although upper leaves at rural B had the lowest N_{leaf} values for this species (2.09 ± 0.20). However, at the urban site D, N_{leaf} was significantly greater in upper than lower leaves for *Q.ilex* (1.36 ± 0.06 L, 1.61 ± 0.03 U

Table 5.4: Site and species differences in sun and shade leaves using leaf position (UPPER and LOWER) to ascertain sun and shade (includes interaction terms). Significant levels are $p < 0.001 = ***$, $p < 0.01 = **$, $p < 0.05 = *$, p $\geq$ 0.05 = not significant (ns).

ANOVA table: Effect of Leaf Position on leaf traits

Response:		SLA		N_{leaf}		C_{leaf}	
	Df	F	P	F	P	F	P
SP	1	1189.75	***	883.85	***	18.23	***
S	5	12.34	***	20.18	***	10.79	***
LP	1	83.70	***	10.11	***	58.18	***
SP:S	5	5.54	***	5.33	***		
SP:LP	1	40.62	***	4.31	*		
S:LP	5	4.41	**	2.11	ns		
SP:S:LP	5	4.61	**				
Residuals	338			146			

(%)(Figure 5.9).

N_{leaf} was significantly higher in *Q. robur* than *Q. ilex* , higher N_{leaf} in upper leaves for *Q. ilex* compared to lower (Table 5.4).

5.4 Discussion.

Although both *SLA* and N_{leaf} were greater in *Q. robur* than *Q. ilex*, C_{leaf} was greater in *Q. ilex*. Differences between species and environment were very site specific, thus highlighting the difficulties in determining species responses when generalising urban environments. The upland site had lowest N_{leaf} in both species and highest *SLA*. In general *SLA* increased from urban to upland environments while N_{leaf} followed the opposite response and generally declined along the same gradient. However, for *Q. ilex*, the site with the highest N_{leaf} was the rural site DT, situated on the outskirts of Totnes town. Other studies also show that *SLA* is higher in sun than shade leaves, and in *Q. ilex* trees, *SLA* decreased with drought (Ogaya and Penuelas 2006).

It has been generalised that deciduous species tend to have higher N_{leaf} than evergreens, coupled with high *SLA* values (e.g. Evans and Poorter 2001; Villar et al. 2006) this has a negative correlation with *RGR* where faster growing species have lower N_{leaf} an higher *SLA* (Villar et al. 2006). However, for phylogenetically related species such as evergreen and deciduous oaks, this relationship does not always hold true and increased leaf mass ratios (LWF_t) in *Q. ilex* can cause this (Antunez et al. 2001). Environmental factors also disrupt this, such as soil fertility,

light availability and the effects of drought (Laureano et al. 2008).

N_{leaf} is tightly coupled with light levels and A_n as much of N within a leaf is responsible for the formation of proteins (around 30%) (Meir et al. 2002) and the enzyme RuBisCO which is responsible for the carboxylation process (Fitter and Hay 2002). Further, $leafN_{mass}$ has been shown to decrease with increasing mean annual temperatures (MAT) coupled with irradiance. Shade leaves therefore tend to have lower mass based N_{leaf} and higher *SLA*. *Q. ilex* saplings tended to have lower mass based N_{leaf} in lower canopy leaves (shade), however, Meir et al. (2002) showed this trend in mature *Q. petrea* therefore the lack of foliage is likely to be responsible for this in *Q. robur*. *Q. ilex* is a Mediterranean evergreen and as such has a conservative growth pattern with regards to leaf traits (Valledares et al. 2000).

Q. ilex growing in the Mediterranean basin had higher N_{leaf} in relation to light despite no effect of nutrient status (Valledares et al. 2000) and also (Ogaya and Penuelas 2007*a*). This was true for my research at the upland site (where mean temperatures were 1-2 $^{\circ}C$ lower than the other sites), and to a lesser extent on the Dartmoor fringe where temperatures were slightly lower. Interestingly this site lies within the east Dartmoor rain shadow (Phillips and Mcgregor 2002) and here both species have higher *SLA* (lower *LMA*) in field grown saplings suggesting that in *Q. ilex SLA* responds to colder temperatures when associated with drought (Ogaya and Penuelas 2007*a*). In fact, *Q. ilex* has more leaves with higher *SLA* in order to maximise photosynthetic gain, when lower temperatures correspond with drought (Ogaya and Penuelas 2007*b*). *SLA* has greater correlation with A_{max} at lower canopy levels as within forest stands this part of the canopy is generally shaded and therefore leaves invest in light harvesting as opposed to photosynthetic enzymes (Meir et al. 2002). Ogaya and Penuelas (2006) also found that *LMA* ($1/SLA$) was higher in sun leaves as *Q. ilex* leaves were thicker with a smaller surface area than a deciduous oak, *LMA* also decreased with drought. Morphologically there is also a gradient from temperate to Mediterranean in *LMA* increase (Ogaya and Penuelas 2006).

In contrast leaves developed in high light environments lead to thick leaves with low *SLA* as leaves develop an extra layer of palisade cells in order to increase the number of chloroplasts and photosynthetic enzymes in order to enhance assimilation rates, of course this comes with a trade

off and light harvesting capabilities are reduced (Evans and Poorter 2001). This coupling means that changes in SLA affect RGR more in low light than high, and although N_{leaf} increased as *SLA* decreased and acclimation to low light results in a twofold increase in *SLA* compared with a 50% reduction in N_{area}. Therefore increasing *SLA* in response to low light is more effective for increasing carbon assimilation than re-allocating N_{leaf} (Evans and Poorter 2001).

5.4.1 Urban Environments.

Adaptations of plants to stress can include changes in morphology such as *SLA*. Decreasing *SLA* is a signature of 'schlerophllous' plants such as *Q. ilex* that are adapted for high temperatures accompany by periods of drought (Filippo 2008).

Generally C_{leaf} was lower in relation to the urban environment while N_{leaf} was higher. *Q. ilex* growing in Naples were found to increase concentrations of both leaf C_{leaf} and N_{leaf} over time even in control trees (Alfani et al. 2000). N_{leaf} from deposited NO_2 is taken up by leaves and rapidly incorporated into nitrogen compounds such as amino acids as a means of removing this gas from the atmosphere (Sparks 2009).

However the stimulation in growth caused by increased N deposition is mediated by water availability and a threshold usually occurs where *N* accumulation starts to have adverse effects on leaf performance and stomatal damage can occur (Abrams 2011). Exposure in my research saplings may not be long enough at the levels measured to induce adverse effects despite increased N_{leaf} at urban sites.

SLA increases with decreasing rainfall (Wright et al. 2004) and *SLA* can decrease with decreasing mean annual temperatures in evergreen broadleaves compared to a more conservative response in deciduous species (Wright et al. 2005). This thesis showed that decreasing *SLA* was more related to lower temperatures and higher nutrient supply, evidenced by the significantly higher *SLA* at the upland site pots. Similarly, Ordpnez et al. (2009) when analysing a global dataset, found low N_{leaf} and low *SLA* corresponded with low soil fertility and vice versa and was related to MAT (Ordpnez et al. 2009). Therefore response in leaf traits of the saplings measured within my research show evidence of complex intrinsic and extrinsic responses in both species, the Mediterranean evergreen increases N_{leaf} at urban locations however this is accented when

nutrient supply is good despite this species in generally having a more conservative response to nutrient supply (Valledares et al. 2000).

5.5 Conclusion.

Q. robur has greater *SLA* and N_{leaf} than *Q. ilex*. This is the general trend in deciduous and evergreen species. Usually greater *SLA* and N_{leaf} corresponds to faster growth in deciduous species. However, young saplings here have shown that this is not always the case. *SLA* seemed to decrease with increasing light in *Q. robur* while, in both species *SLA* increase with decreasing temperatures and in *Q. robur* higher soil nutrients. Low *SLA* is related to increased allocation to photosynthetic process in order to maximise photosynthesis. Therefore decreasing *SLA* with lower N_{leaf} may be a result of photo inhibition in low temperatures and high light environment often seen in Mediterranean oaks and manifest here in deciduous oaks on high nutrient soils.

Chapter 6

Productivity and Biomass Accumulation.

Whole tree allometry is a key area of climate change science historically rooted in commercial forestry science. Essentially, Kyoto Protocol requirements mean Countries need to routinely monitor their carbon stocks therefore providing a renewed interest in biomass determination. The literature contains information on specific allometric equations for species and locations, however, more general equations are also available although their usefulness is debated. This chapter looks at differences in total biomass (TB) and allocation to different root and shoot fractions between the two oak species studied. Survival, and relative growth rates of all saplings remaining until the end of the experiment were determined by analysing monthly growth data taken as height (h) and diameter at ground level (DAG) measurements and expressed as stem volume with the equation $V = HD^2$. An initial harvest was taken of a selection of one year saplings from the control site, these were harvested in September 2009 and initial allometic equations created using TB and HD^2 regression equations. A second harvest was completed at the end of the experimental period September 2011. Ten saplings for each site, species and condition were cut into relative foliage, branch, stem and root fractions and dry weight determined. Differences in TB between species and sites was determined using general linear models and allocation to different fractions was also analysed between species and sites.

Results showed that the evergreen oak not only had faster relative growth rates it also allocated a greater proportion of biomass to leaf and branch fractions in comparison to the deciduous species that allocated more to root and stem.

The evergreen *Q. ilex* competed better at sites where the English oak suffered from drought stress although at the upland site the deciduous leaf habit had the advantage and was able to increase overall biomass at this site to values comparable to *Q. ilex*.

However survival was also higher in the deciduous species at this site. Urban influences were also evident with the deciduous species having lower leaf weight fractions at the urban sites.

Evergreen broadleaved trees are known to increase allocation to foliage when young and this is a competitive strategy that seems to pay off in favourable environments. However as trees mature their relative allocation strategies change and relative leaf weight fraction can decrease to just 6 % of whole tree mass. The urban environment poses many stresses on oak productivity, water limitations and atmospheric pollutants combine to lower water use efficiency and leave them susceptible to long term stress.

6.1 Introduction.

Evidence suggests that European, temperate forests will maintain or increase in productivity with different climate change scenarios; in particular, UK forests have the potential to increase carbon sequestration (Broadmeadow and Matthews 2003). Current carbon sequestering rates for UK forests are estimated at up to 1.1 $GtC\ yr^{-1}$, however, this figure is variable and can depend on how data is collected (Nabuurs et al. 2007). In fact, although more recent eddy covariance[1] methods are useful for analysing ecosystem scale carbon assimilation across biomes, these methods are expensive and large errors can occur (Ohtsuka et al. 2009). Carbon inventory methods using tree allometry are routinely used to determine stem biomass for use in the forestry industry, where stem volume estimates, wood specific gravity, and root shoot ratios or biomass expansion factors (BEF) are used alongside regression to ascertain biomass (Matthews and Mackie 2006; West 2009). Although historically, allometry for different biomass fractions did not play an important part in forestry science, the growing importance of using branch and brash for commercial biomass and the National requirement of carbon accounting for climate change mitigation, mean the science of whole tree allometry is now an important study area (Zianis and Mencuccini 2004).

Further, in the wake of the Kyoto Protocol, Marrakech Accords and subsequent Land-use, Land Use Change and Forestry Guidance (LULUCFG) (IPCC 2003), the UK along with other Coun-

[1] Eddy covariance technique uses infra red gas analyser (IRGA) situated on a tower. This constantly measures the flux of CO_2 between the atmosphere and the canopy. Wind-speed and direction are also monitored and correction factors involving atmospheric conditions used to find CO_2 assimilation rates

tries ratified by the Kyoto Protocol are obliged to monitor carbon stocks and changes at regular intervals (Broadmeadow and Matthews 2004). The Guidance gives advice on sampling protocols to ensure quality reporting of source/sink information including that for(de)(re)(a)fforestation (IPCC 2003).

6.1.1 Carbon Allocation and Relative growth rate.

Direct measurement of biomass alleviates the problem of determining respiration rates when assessing productivity at the smaller scale and can be used alongside NPP studies to ascertain relationships between carbon assimilation and subsequent sequestration. More information on below ground biomass is also desirable: the retrieval of root systems is difficult and time consuming and tends to introduces errors into carbon sequestration modeling (Clark et al. 2001). The destructive harvesting of mature trees is also extremely time consuming and undesirable, yet can be the only way of determining accurate biomass equations for a specific site as more general equations used for simplicity can introduce a wide error range (Clark et al. 2001). In addition to finding whole tree biomass, the relative allocation of carbon to roots, stems, branches and foliage can play an important part in overall carbon sequestration potential of a tree. Evergreen trees are associated with less fertile and drier sites than deciduous and are therefore expected to allocate more carbon to roots than foliage and for deciduous the opposite is true (Litton et al. 2007).

It is also important to note that allocation of assimilated carbon is tightly coupled with mycorrhizal status of a tree (Lunt and Hedger 2003). An exchange of nutrients occurs between plant and fungus and as well as enhancing mineral status within the plant (e.g. Lunt and Hedger 2003) and in some cases improving water use efficiency within the plant (Futai et al. 2008), however the cost to the plant is around 20% of assimilated carbon (Futai et al. 2008).

6.1.2 Allometry and Biomass Fractions.

The ratios of biomass fractions (branch, leaf and root) to stem volume are known as biomass expansion factors (BEF) and multiplication of stem volume by the BEF can provide an estimate for the biomass fraction (West 2009).

Another important aspect of allocation and productivity is ontogeny: For trees, allocation to leaf mass fraction(LMF) is higher in young saplings but as a tree matures its RGR in the stem mass fraction SMF increases as LMF decreases (Poorter et al. 2012). This is logical as a tree grows it stores non living carbon as structural support (Franco 1985) and this quantity is included in biomass estimates. In tree seedlings, the relative allocation patterns of biomass to different aboveground and below ground fractions can change with water, nutrient and temperature stresses (Poorter et al. 2012).

Standard biomass equations for different tree species are often used and there is a plethora of information in the literature on stand level biomass estimates using allometry (e.g. Muukkonen 2007). In the UK, at the national and regional scales the National Inventory of Woodland and Trees (NIWT) has been modified in order to honour the monitoring agreement. The latest Inventory, is due for completion in 2015 (Forestry Commission 2012*b*) and involves an extensive monitoring network. This network includes mapped information on soils, species composition, productivity, structure and management of UK forests. This is being 'backed up' by three more monitoring networks at various detail and scales (Forestry Commission 2012*a*). Crucially, assessment of productivity and carbon sequestration potential in species in relation to environment is important not only to help monitor stocks already established but to assess productivity of non-native species that may become more frequent with future climate change. Further, the increased occurrence of damaging pathogens in UK woodlands mean that native species are becoming increasingly at risk and may not have the ability to out-compete those species moving in.

Generally biomass equations take the form of the power function:

$$M = aD^b \tag{6.1}$$

where M is mass and D, the diameter at breast height, while a and b are scaling coefficients usually determined via least squares linear regression on log transformed data (to avoid issues with normality and misuse of power functions) (Zianis and Mencuccini 2004). Although information regarding different scaling coefficients and variations of the above equation is abundant

in the literature, the values are very much species and site specific, despite attempts to simplify and generalise equations for use with different forest types (Zianis and Mencuccini 2004).

Volume estimates, using height and diameter at breast height (DBH), for trees with $DBH > 7cm$ are the normal method of estimating biomass content in commercial forestry (Matthews and Mackie 2006). For seedlings and young saplings diameter at ground level (DAG) can be used as an alternative to DBH and, incorporated into a stem volume equation as HD^2, has been proven to be useful for measuring biomass in young hardwood species in Central America (Williams and McClenahen 1984) (see eqn 6.2). Although Williams and McClenahen (1984) and others (e.g. Zianis and Mencuccini 2004) suggest removal of H from the equation to simplify fieldwork, other research suggests errors involved in estimating biomass without H can be too large to warrant its removal (e.g. Ketterings et al. 2001). Additionally, for seedlings and young saplings, height may be an important variable when measuring growth as height growth tends to be more rapid during early growth stages compared to that of a mature tree (Grime 2002), indeed, Canadell et al. (1988) suggest it is useful addition in biomass estimation. Therefore for the purpose of finding allometric equations for *Q. ilex* and *Q. robur* growing at six different sites at various rural, urban and one upland location, the logged form of the power function D^2H was used (in order to homogenise variances) and using DAG as a substitute for DBH). The natural Log_e (Ln) was used and once allometric equations determined, a correction factor (CF) added in order to increase reliability of the equation when back transforming data into the power function. When mean square errors of the regression are small, equation 6.5 has been proven to be a reliable CF for biomass regression (Baskerville 1972; Mascaro et al. 2011).

$$V_i = D^2H \tag{6.2}$$

$$Log_eY = a + bLog_e(D^2H) \tag{6.3}$$

$$Y = a(D^2H)xCF \tag{6.4}$$

$$CF = e^{(MSE/2)} \tag{6.5}$$

where Y is biomass and a and b are scaling coefficients and CF is the correction factor and MSE is the mean square error of the regression.

The experiments in this chapter were designed to assess productivity and biomass accumulation of each species in relation to the relative urban, rural and upland environments. Actual growth and relative growth rates (RGR) have been determined for growing season and deciduous dormant season, overall growth rate for the 30 month period and also a the smaller scale of approximately growth every two months. Growth was measured as HD^2 and $\overline{RGR}$ has been determined using the standard $\overline{RGR}$ equation presented in Hunt (1982) (see eqn. 6.6, page 97, this chapter). To standardize this equation and reduce error, data were logged before means were taken and not after (see Hoffman and Poorter (2002)). Allometric equations for biomass estimates were determined using final HD^2 and final harvest (TB and AGB) with least squared regression on Log_e transformed data. Specific density was not found and therefore has not been used as an additive parameter due to the young age of the trees; average specific density in the literature may not be accurate. Specific gravity for mature oaks can range from 0.4-0.8 with an average found for white oak growing in the Southern Appalachian mountains of 0.6 (Clark III and Schroeder 1986).

General working hypotheses for this chapter are:

1. Working hypotheses

    1.1. For *Q. ilex*, mean $\overline{RGR}$ and actual growth is significantly faster than *Q. robur*.

    1.2. For *Q. ilex* growth is faster than *Q. robur* at each of the six sites.

    1.3. Is final total biomass and carbon content greater in *Q. ilex* than *Q. robur* and is this true for each site.

    1.4. Finally is there any significant affect of urban and rural as a site character or are any differences site specific.

6.2 Methods.

Initial height (H) and ground level diameters (DAG) were recorded at each site after planting in February 2009, then height and diameters recorded every 1 - 2 months until final harvest in August/September 2011 (see chapter 2 section 2.3 on page 21 for detailed sampling protocol). Sapling deaths within the first (Feb 09- Nov 09) second (Nov 09- Nov 10) and third (Nov 10- Aug 11) season were recorded and shown as percentage losses. Dead saplings were deemed those showing no evidence of green shoots at the end of the favourable season (1st year losses were determined at the beginning of the second favourable season in case of re-growth). Actual and *RGRs* are shown using data from those saplings surviving the entire 30 month growth period (see 6.3).

6.2.1 Relative growth Rates.

The natural log_e (*Ln*) was used to normalise data as $Ln(HD^2)$. Subsequently, $Ln(HD^2)$ was used to signify growth from all sites and used to compare growth rates using the standard equation for $\overline{RGR}$ but revised to substitute $ln(HD^2)$ in place of weight (W) (see equation 6.6). In addition to this, *Ln* values of HD^2 were used to compare *RGR* between Feb 2009 - Sep 2009, Sep 2009 - Feb 2010, Feb 2010 - Sep 2010, Sep 2010 - Feb 2011, Feb 2011 - Aug 2011 (final harvest).

$$\overline{RGR} = (\overline{Ln(HD^2)_2} - \overline{Ln(HD^2)_1})/({}_2t - {}_1t) \tag{6.6}$$

During September 2009 (7 months after planting) a small number of saplings were harvested ($harvest_1$). Due to the number of losses in the first growing season this sample was taken from a sub-sample of original planted saplings at the control site and not from the individual sites (again see chapter 2 section 2.3 on page 21 for detailed sampling protocol). Due to this, $harvest_1$ data was used to provide base line allometric equations for each species but was not included in any *RGR* analyses (HD^2 was used instead - see above). Approximately 10 saplings per treatment (depending on surviving saplings at each site) were harvested at the end of the experiment September 2011 and total biomass of each fraction determined using the methods described in chapter 2. Final harvest data was used to determine overall carbon sequestered over the 30

month period and also to see if there were any differences between species in allocation between the fractions at each site. Finally, least squares regression was used to compare relationships between HD^2 with AGB and TB and site specific allometric equations established.

Statistical analyses were only performed on those saplings that had survived throughout the entire experiment. Descriptive statistics showing initial H and DAG measurements and statistical differences between sites for each species and parameter are shown (under Group), followed by final biomass as separate fractions and above ground biomass (AGB) and total biomass (TB), H and DAG measurements. Comparisons between sites for final biomass are shown using naturally logged data (ln) data, for homogenised variances. General linear models (GLM) were performed on the logged data to look for the effects of site and species plus interactions on growth parameters. Allocation to the different biomass fractions were analysed for effects of different sites and species to ascertain the importance of each fraction to overall biomass at each site (see appendix B for residual plots).

Statistics software used was 'R' for GLM's and simple least squared regression for allometric equations (R 2009), descriptive statistics were generated with Minitab16 (Minitab ® 2010).

6.3 Results.

6.3.1 Survival.

The effect of the sampling design meant that there were more losses at the outer edge of the blocked saplings, although this was most evident in *Q. ilex* saplings at the exposed sites at rural, B on the Dartmoor fringe and urban L, on the south facing slope near the city center. Most losses occurred in the first growing season while saplings were still acclimatising and *Q. ilex* lost more saplings from the upland site than *Q. robur* in both soil conditions and also lost more saplings over the three seasons at the rural site B and the urban site D in potted trees (Table 6.1). Although *Q. robur* had greater survival than *Q. ilex* in general, at the exposed urban site L, field grown *Q. robur* suffered the greatest loses, due to exposure and increased drainage at the top of the slope increasing the occurrence of drought (Table 6.1).

Table 6.1: Cumulative percentage of lost saplings over 3 growing seasons for Upland site (DPF), rural sites at Dartington (DT) and Broadley (B) and urban sites at Digginit (D) and Laira (L).

Accumulative percentage losses (%) of Saplings over three years

Character	Site	Condition	Sep-09	Sep-10	Sep-11	Sep-09	Sep-10	Sep-11
			Q. ilex % loss			*Q. robur* % loss		
	GROUND		%	%	%	%	%	%
Upland	DPF		15	35	50	10	15	15
Rural	B		25	25	30	35	35	35
	DT		15	20	20	40	40	40
Urban	D		35	40	40	30	40	45
	L		45	45	45	80	80	80
	POTS							
Upland	DPF		50	55	65	30	40	40
Rural	B		40	50	50	15	25	35
	DT		25	25	25	15	20	20
Urban	D		45	50	50	25	30	35
	L		50	50	55	0	0	5
Control site	CS		15	20	20	30	30	30

6.3.2 Initial height and diameters.

One year saplings were checked for significant differences, in H and DAG, between sites at start of growth period (February 2009). Data was log transformed (using the natural log ln)and Levenes test was used to determine homogeneous variances. Levenes test showed that variances in both $ln(H)$ and $ln(DAG)$ datasets were homogeneous ($T = 0.65 p = 0.751; 1.12, p = 0.344$). One-way ANOVA showed no significant differences between sites for mean DAG or mean H for *Q. robur* ($p > 0.05$). However for *Q. ilex*, initial heights and diameters were more variable and *Q. ilex*, mean DAG was significantly lower at the rural site B ($F_{(4,77)} = 8.64, p < 0.001$). Subsequently, Tukeys test showing significant differences in H between sites B and CS ($F_{(4,77)} = 3.04, p < 0.05$) (CS saplings were generally smaller in H) (see Table 6.2).

6.3.3 Actual Growth and Relative Growth rates.

Figure 6.1 shows actual growth for saplings over the entire experimental period, using stem volume HD^2 as the measure of growth. At the control site (CS) *Q. ilex* had the greatest overall growth (Figure 6.1 but see appendix E, section E.1 for individual growth graphs) (around 2000g

Table 6.2: Descriptive statistics for height (H) and diameter at ground level (DAG) measurements taken at planting in February 2009 means, stadnard deviations (StDev, minimum and maximums given. Different letters denote significant differences between sites; Rural sites, Broadley B, Dartington DT, control site CS, upland site DPF and urban sites Digginit D and Laira L.

Descriptive statistics for initial height (H) and diameter at ground level (DAG)measurements

Variable		SITE	N	Mean	StDev	Minimum	Maximum	Group
				Q. robur				
H	RURAL	B	19	209.05	37.91	159	293	A
		DT	19	203.21	28.19	167	260	A
	CONTROL	CS	7	162.3	46.9	105	223	A
	UPLAND	DPF	29	217	42.55	168	360	A
	URBAN	L	15	223.6	54.4	175	390	A
		D	20	201.9	66.4	105	418	A
DAG	RURAL	B	19	4.313	0.91	2.815	6.34	A
		DT	19	4.379	0.706	3.31	5.86	A
	CONTROL	CS	7	4.531	0.976	3.42	5.95	A
	UPLAND	DPF	29	4.603	0.945	3.18	7.1	A
	URBAN	L	15	4.365	0.889	3.04	6.18	A
		D	20	4.751	1.654	2.64	10.09	A
				Q. ilex				
H	RURAL	B	19	261.47	39.01	187	350	A
		DT	17	248.4	42.9	185	330	AB
	CONTROL	CS	8	205.5	17.25	186	230	B
	UPLAND	DPF	17	259.94	37.56	200	335	A
	URBAN	L	15	255.4	30.86	200	305	AB
		D	14	235.4	52.3	129	280	AB
DAG	RURAL	B	19	3.692	0.753	2.62	5.08	C
		DT	17	4.419	0.818	3.3	6.49	AB
	CONTROL	CS	8	5.158	0.45	4.54	5.76	A
	UPLAND	DPF	17	4.691	0.77	3.29	6.34	AB
	URBAN	L	15	4.225	0.587	3.17	5.45	BC
		D	14	5.048	0.501	4.37	5.76	A

higher average stem volume than CS *Q. robur*) saplings. Rural site B on the Dartmoor fringe showed the greatest growth in *Q. ilex* in both potted and field grown saplings, however at the more favourable rural site DT on the outskirts of Dartington, both species have higher HD^2 in field grown saplings than pots (Figure 6.1D and E.4). In fact, when actual height and diameters were used to model growth, *Q. ilex* grew faster at all sites except the upland site on the high moor and in planted saplings at DT where *Q. robur* was able to maintain similar growth (Figure 6.1).

Relative growth rates show that for potted saplings, *Q. ilex* maintained higher total $\overline{RGR}$ at rural site B and at CS (Figure 6.21). However this advantage is greater in field grown saplings (Figure 6.22). total $\overline{RGR}$ is only higher in *Q. robur* at the upland site DPF (Figure 6.2). Appendix E also gives $\overline{RGR}$ data for seasonal growth and shows that *Q. ilex* has greater $\overline{RGR}$ during the first growing season (Feb 09 - Oct 09), however, after establishment (one year), *Q. robur* increased its growth rate at all sites until $\overline{RGR}$ was similar to *Q. ilex* during the final growth period (Apr 09 - Aug 11) at all sites (Figures E.7 to E.12 in appendix E). In fact field grown saplings generally had faster $\overline{RGR}$ than potted saplings although for *Q. robur* planted at the upland site this was not true. Overall, $\overline{RGR}$ were lower at the rural site B and upland DPF for *Q. robur* and higher at D and DT. $\overline{RGR}$ in *Q. ilex* remained constant across sites apart from the upland site where $\overline{RGR}$ was as low as 0.004 cm^3t^{-1} (Figure 6.2) (Appendix E gives figures for monthly and seasonal $\overline{RGR}$s).

6.3.4 Final growth and Biomass.

TB was greater in *Q. ilex* containing over twice as much biomass than than *Q. robur* (284.2 $\pm$ 259.85; 119.5 $\pm$ 134.3, mean $\pm$ SD) and thus proving the first hypotheses. There were no differences between potted (P) and field grown (G) saplings for the majority of sites, however, P had higher TB than G at the upland site and lower TB at the rural site DT (see tables 6.3, E.1 and E.2). *Q. robur* maintained similar TB at the upland site, where both species had reduced biomass (33.62 $\pm$ 53.6; 39.7 $\pm$ 59.5 for *Q. ilex* and *Q. robur* respectively). The highest TB was found at DT and D, both species maintained their highest growth and biomass at these sites and *Q. ilex*, P experiencing the highest TB at urban D site (632.72 $\pm$ 374.96), twice as much as the

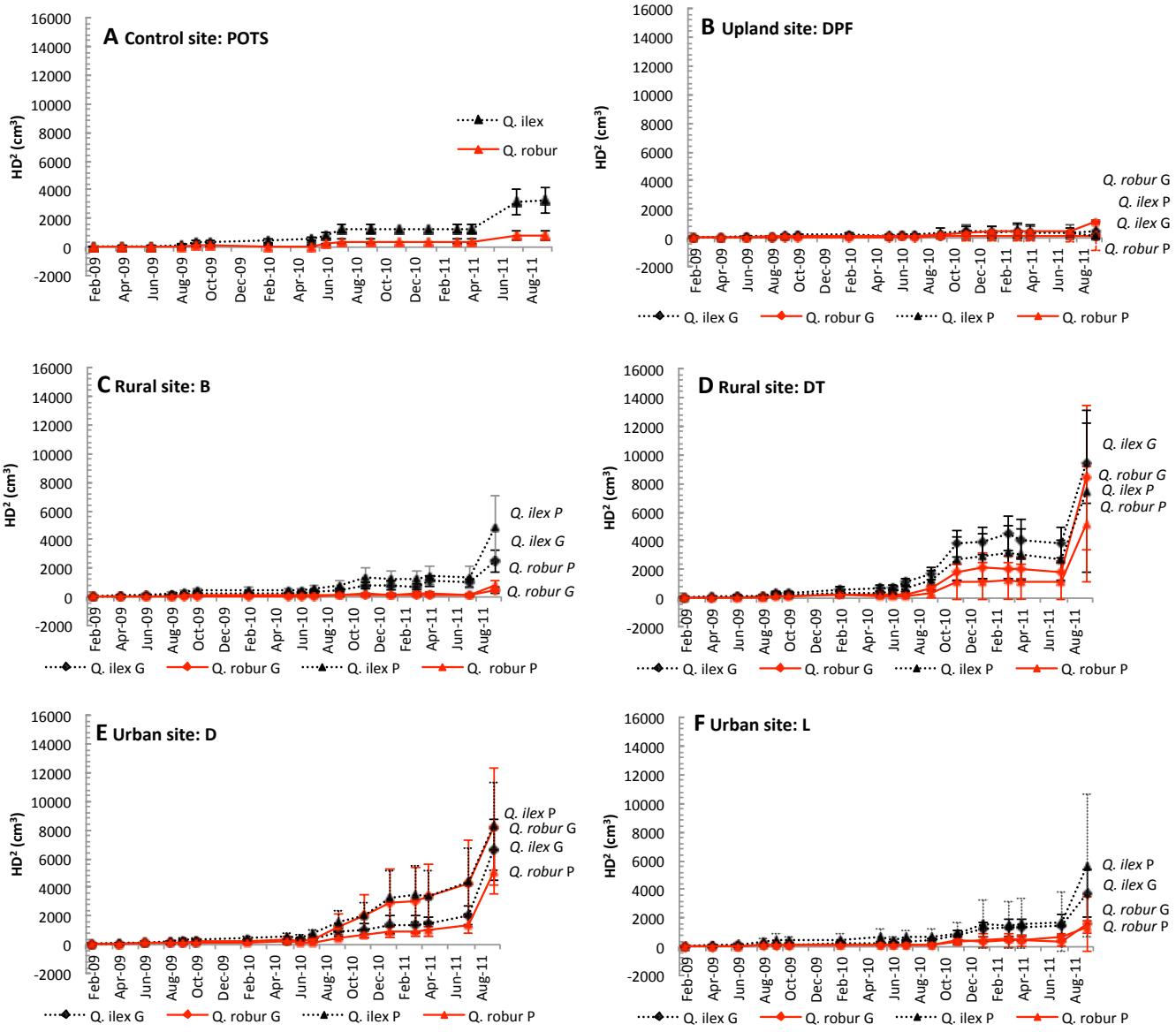


Figure 6.1: Actual growth; **A**: Control site CS, **B**: Upland site DPF, **C**: Rural site B, **D** Rural site DT, **E** Urban site D, and **F** Urban site L (monthly means ± 95 % CI).

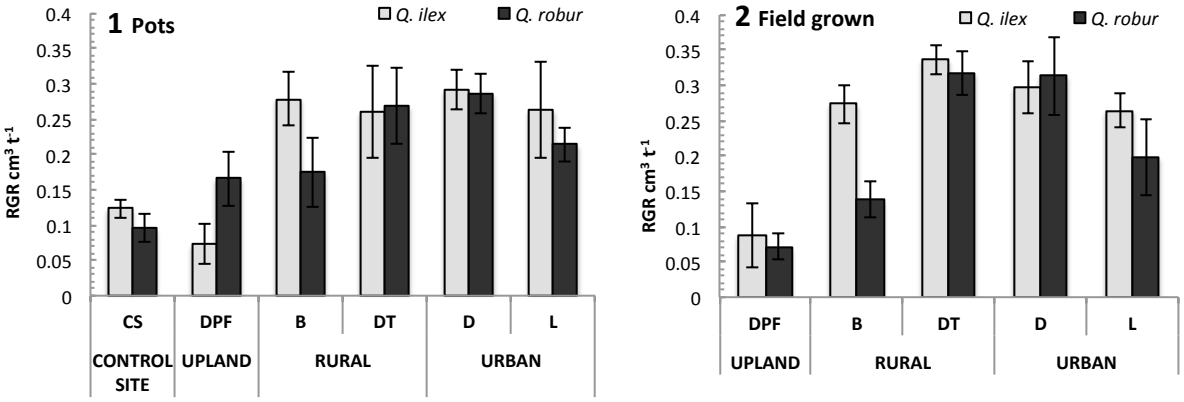


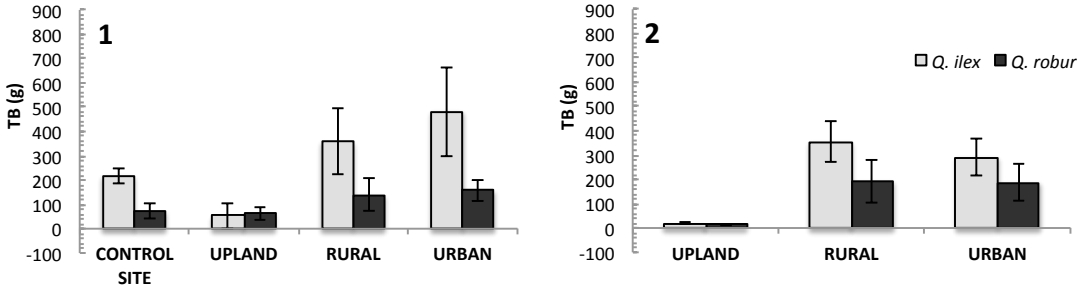
Figure 6.2: Relative growth rate (RGR) for all saplings between planting in Feb 09 and final harvest Aug 11 using eqn 6.6. Rural sites, Broadley B, Dartington DT, control site CS, upland site DPF and urban sites Digginit D and Laira L (means $\pm$ 95 % CI).

Table 6.3: Species, site and soil treatment (pots (P) or field grown (G) saplings) effects on total biomass, Degrees of freedom for factors and residual errors (Df), F statistic and significance levels given as (sig. p<0.05 '*', p<0.01 '**' p<0.001 '***' and p $\geq$ 0.05 not significant 'ns')

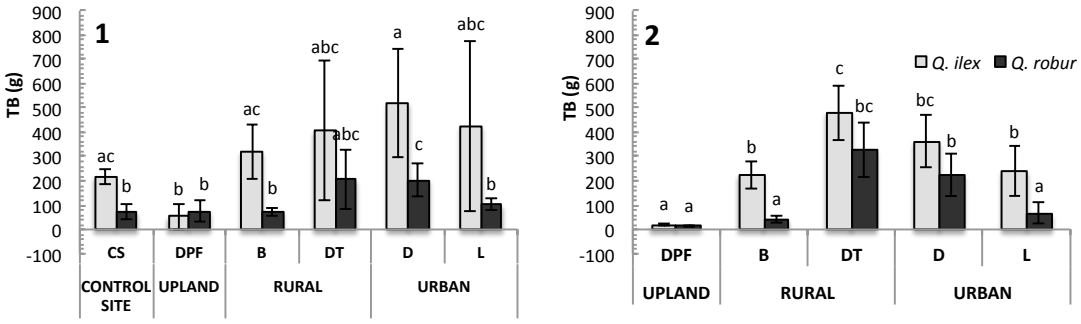
ANOVA table: Total Biomass (TB)			
Variable		TB (g)	
Factors	Df	F	sig.
SITE	4	84.8514	***
SPECIES	1	45.0447	***
TREATMENT	1	7.3847	**
SITE:SPECIES	4	6.8617	***
SITE:TREATMENT	4	7.2169	***
SPECIES:TREATMENT	1	1.0208	ns
SITE:SPECIES:TREATMENT	4	0.6284	ns
Residuals	162		

G saplings for this species (Tables E.1 and E.2). Interestingly, TB in *Q. robur* at this site was similar for both P and G saplings and along with DT, D was *Q. roburs* better site. However, DT field grown saplings had the greatest TB (327.842 $\pm$ 186.524) in *Q. robur*.

Both species had lower biomass at rural B and urban L although again *Q. ilex* is still over twice as much as *Q. robur* (Table E.1 and E.2). There is a huge amount of variation in the data, and differences in biomass is very site specific, to show this, when data was analysed according to site character where rural and urban sites were analysed together. There was a significant effect on TB from character and species ($p < 0.05$) (data not shown). However, this was due to the reduced biomass at the upland site and there were no significant differences between rural and urban sites. Therefore data shown is for SITE, SPECIES and interactions for G and P saplings (Tables E.1 and E.2).



(a) Environmental differences in total biomass (TB g) for upland, rural and urban environments.



(b) Site differences in total biomass (TB g)

Figure 6.3: Environmental and site specific differences in total biomass for 1) potted and 2) field grown saplings (means $\pm$ 95 % CI). Rural sites, Broadley B, Dartington DT, control site CS, upland site DPF and urban sites Digginit D and Laira L.

Table 6.4: ANOVA table for biomass and allocation, all variables analysed in *ln* form; Total biomass TB (g); root weight fraction (RWF_t) leaf weight fraction (LWF_t), stem weight fraction (SWF_t) and branch weight fraction (BWF_t) analysed as percentage of total biomass. Factors are, SITE: upland DPF, rural B and DT and urban D and L; TREATMENT: soil treatment, POT (G) or field grown (G). Significance values; sig. p<0.001 '***', p<0.01 '**', p<0.05 '*', p$\geq$ 0.05 not significant 'ns'.

Response variable		$ln(TB)$		$ln(RWF_t)$		$ln(LWF_t)$		$ln(SWF_t)$		$ln(BWF_t)$	
FACTOR	Df	F	sig.	F	sig.	F	sig.	F	sig.	F	sig.
SITE	4	84.85	***	17.14	***	3.15	**	10.12	***	37.75	***
SPECIES	1	45.04	***	38.10	***	20.83	***	0.27	ns	40.73	***
TREATMENT	1	7.38	**	12.02	***	2.13	ns	16.60	***	3.81	ns
SITE:SPECIES	4	6.86	***	2.47	ns	2.03	ns	3.33	**	5.02	***
SITE:TREATMENT	4	7.22	***	1.53	ns	0.60	ns	0.81	ns	5.73	***
Residual error	162										

6.3.5 Allocation to Biomass Fractions.

Q. robur allocated a greater percentage of its total biomass (TB) to the root fraction (RWF_t) (37.31 $\pm$ 8.91; 47.62$\pm$ 5.94 g) ($p < 0.05$) and also allocated more to stem (SWF_t) than *Q. ilex* (Table E.1 and Table 6.4). However, *Q. ilex* branch and foliage allocation (BWF_t and LWF_t) increased overall biomass in this species (Tables E.1 and E.2) (*Q. ilex* allocated 24.8 $\pm$ 8.91 g compared to 18.03 $\pm$ 5.94 in *Q. robur*) to LWF_t and BWF_t combined. *Q. robur* allocated just over half the amount of its LWF_t to BWF_t whereas, *Q. ilex* allocated $2/3rd$ of its LWF_t to BWF_t. Again site specific differences in allocation were evident. Both species allocated more to RWF_t at the upland site, and *Q. robur* also allocated greater biomass to the RWF_t at the urban site L. As expected greater RWF_t occurred in P, however, no interactions occurred with this factor (Tables E.1 and E.2). LWF_t was higher in *Q. ilex* than *Q. robur*. Only the upland site showed no significantly higher allocation to LWFt in *Q. ilex* (See appendix E, tables E.1 and E.2 for descriptive statistics and for more information on differences see Table 6.4 and appendix C for interaction plots).

6.3.6 Allometric Equations for Biomass Estimation.

The following tables 6.5 to 6.9 give regression coefficients, associated errors and adjusted R^2 and include the CF for reverting estimated biomass from logged to original form. Table 6.5 gives information for $harvest_1$ regressions, and Table 6.6 on page 108 gives information on equations generated on control site and grouped datasets for $harvest_2$. Table 6.7 on page 109

gives equations for upland site TB and AGB and tables 6.8 and 6.9 on pages 110 and 111 give information from regression at rural and urban sites for $harvest_2$.

Appendix E, Figures F.1, F.2, F.3 and F.4 on pages 206,207, 208 and 209 show HD^2 TB and AGB scatter for individual factors and for species.

6.3.7 Initial Harvest.

Using ANCOVA in R to determine the added effects of species to the linear regression in initial harvest data, no extra significance was brought by analysing the species separately, (p>0.05), therefore only regression equations for combined species are given (Table 6.5).

Significant correlations are evident between coefficient of the slope of the regression and the coefficient of the independent variables for AGB however for TB the coefficient of the independent variable dose not meet the assumptions for a significant correlation at the 95% confidence interval. However the overall regression is significant and has some predictive power despite a low adjusted R^2 (69%) (Table 6.5).

6.3.8 Final Harvest.

For the final $harvest_2$, allometric equations for total dataset and species datasets show significant slope coefficients (a) and also independent variable coefficient b, adjusted R^2 for grouped data was between 84 and 89 % for TB and AGB data (Table 6.6).

When analysed separately for each factor level, predictive power of the equations is reduced for control site saplings (Table 6.6). *Q. robur* $adjR^2$ only 49.3%. The sites where trees were the most stressed, equations seemed to lose predictive power with *Q. ilex* saplings at DPF upland site showing very little individual allometry (Table 6.7). Interestingly despite HD^2 being generally better at predicting AGB, at the upland site it was better at predicting TB, probably due to the increased $RWFt$ at this site. In some cases reduced sapling numbers led to high adjusted R^2 values, however, due to the reduced sample numbers at these sites these equations are not robust enough (e.g. LRG, Table 6.9). In most cases *Q. robur* pots showed lowest prediction (adj R^2 values less than 60%), however, contrary to this upland and DT had good predictive power ($adjR^2 > 90\%$). HD^2 was better at predicting TB in field grown G saplings, although for *Q.*

Table 6.5: Multiple regression on initial harvest data in September 2009 for aboveground biomass (AGB) total biomass (TB), scaling coefficients *a* and *b*, variable SE, MSE and CF shown. Significant levels are; p≥0.05 'ns', p<0.05 '*', p<0.01 '**', p<0.001 '***'.

Growth parameter	Coefficient symbol	value	Variable SE	sig	MSE $ResSE^2$	CF $e^{(MSE/2)}$	$adjR^2$ (%)	overall sig.
AGB	*a*	-2.360	0.320	***	0.133	1.069	91.40	***
	b	0.860	0.061	***				
TB	*a*	-1.063	0.570	ns	0.428	1.239	69.40	***
	b	0.740	0.109	***				

robur DPFRP had better predicitive power than DPFRG reduced R^2 values (Tables 6.7, and 6.8, for scatterplots see Figures F.1 and F.2).

6.4 Discussion.

6.4.1 Relative growth rates and biomass allocation.

Relative growth rate.

My research has shown that in well watered and mild conditions such as that found at the control site. The evergreen oak *Q. ilex* has the advantage over the deciduous *Q. robur* in terms of faster $\overline{RGR}$. However, this took into account the deciduous winter period when *Q. robur* was not in leaf. Looking at $\overline{RGR}$ on a two monthly basis the English oak increased its $\overline{RGR}$ during spring, summer and in the final year in early spring while not in leaf. This growth strategy is common in ring porous oaks, where radial stem growth is added in preparation for new leaves and early wood formation of large transport vessels (Barabaroux and Beda 2002; Gieger and Thomas 2002). In the past it has been generalised that deciduous species have faster *RGR* than evergreens due to generally higher carbon assimilation rates and higher *SLA* (e.g. Wright et al. 2004). However, when taking phylogenetic relationships into account Antunez et al. (2001) found that this was not always true, and in closely related evergreen and deciduous oak species, the evergreen Cork oak (*Q. suber*) had similar *RGR* as deciduous *Q. robur*. This was attributed to the greater *LMF* in relation to its lower *SLA* which in turn masked differences in the ratio between leaf area and plant mass; leaf area ratio (*LAR*) ; In fact, in agreement with the general trend, the same study showed *Q. ilex* subsp. *rotundifolia* had lower *RGR* than *Q. robur* (13.1

Table 6.6: Allometric relationship between HD^2 and TB/AGB. Variables shown, Complete dataset, individual species complete set and Control site groups Showing; Variable Std error, Mean Sq Error (residual) (MSE) and Correction factor CF for allometric regressions (significance levels shown are p<0.001 '***', p<0.01 '**', p<0.05 '*', p>0.05 'ns'.). All control site saplings = 'CS', *Q. ilex* control site saplings = 'CIP' and *Q. robur* control site saplings = 'CRP'.

Regression coefficients: $ln(M) = a + bln(HD2)$

CONTROL AND GROUPS							
Sample	Coefficient symbol	value	Variable SE	sig	MSE $ResSE^2$	CF $e^{(MSE/2)}$	$adjR^2$ (%)
			TOTAL BIOMASS				
All sites/species	*a*	-0.911	0.164	***	0.223	1.118	85.98
	b	0.761	0.022	***			
All *Q. ilex*	*a*	-0.888	0.281	**	0.236	1.125	84.15
	b	0.776	0.036	***			
All *Q. robur*	*a*	-0.662	0.190	***	0.176	1.092	86.90
	b	0.707	0.027	***			
CONTROL Site							
CS	*a*	2.891	1.252	*	0.077	1.04	86.5
	b	0.687	0.072	***			
CIP	*a*	8.603	3.981	ns	0.047	1.02	21.21
	b	0.372	0.219	ns			
CRP	*a*	0.979	3.795	ns	0.118	1.06	64.8
	b	0.803	0.232	*			
			ABOVEGROUND BIOMASS				
All sites/species	*a*	-2.230	0.177	***	0.258	1.138	87.12
	b	0.860	0.024	***			
All *Q. ilex*	*a*	-2.061	0.298	***	0.265	1.142	85.50
	b	0.865	0.038	***			
All *Q. robur*	*a*	-1.958	0.191	***	0.177	1.093	89.30
	b	0.795	0.027	***			
CONTROL Site							
S	*a*	-0.626	1.739	ns	0.149	1.077	83.57
	b	0.851	0.100	***			
CIP	*a*	5.009	2.884	ns	0.025	1.012	60.21
	b	0.541	0.159	*			
CRP	*a*	-3.163	6.313	ns	0.327	1.178	49.29
	b	1.006	0.385	*			

$\pm$ 2.7; 19.2 $\pm$ 2.2 $mg\ g^{-1}$) (Antunez et al. 2001). A later study, also confirmed higher *RGR* in deciduous versus evergreen oaks, where *LAR* was higher for *Q. ilex*, and *SLA* was higher in the deciduous *Q. faginea*, the deciduous species having twice as much biomass at end of the growth period (Ruiz-Roberto and Villar 2005).

In favourable environments *Q. robur* was able to increase $\overline{RGR}$ to rates comparable and in some

Table 6.7: Allometric relationship between HD^2 and TB/AGB for Upland data.Showing; Variable Std error, Mean Sq Error (residual) (MSE) and Correction factor CF for allometric regression equations (significance levels shown are p<0.001 '***', p<0.01 '**', p<0.05 '*', p>0.05 'ns'). All upland site saplings = 'DPF', all *Q. ilex* upland site saplings = 'DPFI', all upland site *Q. robur* saplings = 'DPFR', for field grown saplings a 'G' is added to the sample code and for potted saplings 'P'.

Regression coefficients: ln(M) = a+bln(HD2) for Upland site (DPF)

Sample	Coefficient symbol	value	Variable SE	sig	MSE $ResSE^2$	CF $e^{(MSE/2)}$	$adjR^2$ (%)
			TOTAL BIOMASS				
DPF	*a*	2.70	1.39	ns			
	b	0.670	0.089	***	0.395	1.22	55.2
DPFI	*a*	10.37	3.758	*			
	b	0.179	0.245	ns	0.671	1.40	-2.99
DPFR	*a*	-0.146	0.819	***			
	b	0.845	0.052	***	0.101	1.05	90.3
DPFIP	*a*	13.9	12.178	ns			
	b	-0.032	0.802	ns	1.13	1.76	-16.6
DPFIG	*a*	8.349	2.324	**			
	b	0.291	0.150	ns	0.21	1.11	25.6
DPFRG	*a*	4.027	1.839	*			
	b	0.566	0.122	***	0.09	1.05	58.0
DPFRP	*a*	-1.184	1.175	ns			
	b	0.910	0.071	***	0.08	1.04	93.7
			ABOVEGROUND BIOMASS				
DPF	*a*	1.50	1.507	ns	0.466	1.262	53.30
	b	0.701	0.097	***			
DPFI	*a*	9.56	4.05	*	0.781	1.478	-3.23
	b	0.187	0.264	ns			
DPFR	*a*	-1.543	0.927	ns	0.129	1.067	88.94
	b	0.887	0.059	***			
DPFIP	*a*	14.957	12.906	ns	1.268	1.885	-16.07
	b	-0.150	0.850	ns			
DPFIG	*a*	7.319	3.020	ns	0.350	1.191	16.66
	b	0.315	0.195	ns			
DPFRG	*a*	2.578	2.169	ns	0.127	1.066	51.52
	b	0.611	0.144	**			
DPFRP	*a*	-2.256	1.343	ns	0.102	1.052	92.30
	b	0.934	0.081	***			

cases higher than *Q. ilex* where temperatures and soil nutrients were favourable (Figures E.7 to E.12 in appendix E give $\overline{RGR}$s for saplings growing in two monthly growth increments). The increased $\overline{RGR}$ seen during the last growth phase, includes the most favourable season (April 2011 - August 2011) and confirms that growth in deciduous oak was faster than *Q. ilex* between early spring and summer at sites where conditions were favourable. However, as *Q. ilex* was

Table 6.8: Allometric relationship between HD^2 and AGB/TB. Variables shown: RURAL. Variable Std error, Mean Sq Error (residual) (MSE) and Correction factor CF for allometric regression equations (sig: $***p < 0.001, **p < 0.01, *p < 0.05, nsp > 0.05$). All rural site saplings = 'DT' or 'B', all *Q. ilex* rural site saplings = 'DTI' or 'BI', all rural site *Q. robur* saplings = 'DTR' or 'BR', for field grown saplings a 'G' is added to the sample code and for potted saplings 'P'.

Regression coefficients: $ln(M) = a + bln(HD^2)$

		TOTAL BIOMASS						ABOVEGROUND BIOMASS					
Sample	Coefficient symbol	value	Variable SE	sig	MSE $ResSE^2$	CF $e^{(MSE/2)}$	$adjR^2$ (%)	value	Variable SE	sig	MSE $ResSE^2$	CF $e^{(MSE/2)}$	$adjR^2$ (%)
						RURAL							
B	*a*	1.78	0.837	*	0.135	1.07	86.5	-1.15	0.887	ns	0.151	1.08	88.8
	b	0.757	0.049	***				0.891	0.052	***			
BI	*a*	2.346	1.530	ns	0.067	1.03	80.12	-0.109	1.611	ns	0.075	1.038	82.75
	b	0.731	0.085	***				0.839	0.090	***			
BR	*a*	4.626	1.705	*	0.171	1.09	61.45	1.861	1.793	ns	0.189	1.099	68.17
	b	0.575	0.106	***				0.698	0.111	***			
BIP	*a*	3.07	2.267	ns	0.08976	1.05	78.73	-1.237	1.785	ns	0.056	1.028	91.11
	b	0.689	0.125	***				0.893	0.098	***			
BIG	*a*	-0.258	2.66	ns	0.055	1.03	78.79	-1.76	2.94	ns	0.067	1.03	77.52
	b	0.879	0.150	***				0.938	0.166	***			
BRG	*a*	2.95	2.080	ns	0.096	1.05	75.93	0.248	2.047	ns	0.092	1.05	82.08
	b	0.668	0.130	**				0.787	0.128	***			
BRP	*a*	6.897	2.21	*	0.167	1.09	52.31	4.055	2.511	ns	0.216	1.114	58.96
	b	0.447	0.136	*				0.574	0.154	***			
DT	*a*	2.543	1.02	***	0.115	1.06	82.37	-0.058	1.171	ns	0.151	1.079	82.85
	b	0.703	0.055	***				0.822	0.063	***			
DTI	*a*	-0.352	0.951	ns	0.040	1.02	94.75	-3.040	0.944	**	0.040	1.020	96.01
	b	0.864	0.051	***				0.990	0.050	***			
DTR	*a*	5.14	1.349	***	0.116	1.06	75.94	2.802	1.570	ns	0.157	1.081	76.55
	b	0.557	0.073	***				0.658	0.085	***			
DTIP	*a*	-0.871	1.459	ns	0.067	1.03	95.41	-3.324	1.224	*	0.047	1.024	97.40
	b	0.894	0.080	***				1.004	0.067	***			
DTIG	*a*	1.257	2.099	ns	0.029	1.01	84.49	-1.020	2.461	ns	0.040	1.020	83.61
	b	0.779	0.110	***				0.885	0.129	***			
DTRP	*a*	5.65	1.41	**	0.085	1.04	84.49	3.053	1.182	*	0.060	1.030	92.12
	b	0.520	0.078	***				0.633	0.065	***			
DTRG	*a*	6.09	2.69	ns	0.123	1.06	56.5	5.225	3.28	ns	0.184	1.10	48.3
	b	0.513	0.144	***				0.538	0.176	*			

Table 6.9: Allometric relationship between HD^2 and AGB/TB. Variables shown: URBAN. Variable Std error, Mean Sq Error (residual) (MSE) and Correction factor CF for allometric regression equations (sig: $***p < 0.001, **p < 0.01, *p < 0.05, nsp > 0.05$). All urban site saplings = 'D' or 'L', all *Q. ilex* urban site saplings = 'DI' or 'LI', all urban site *Q. robur* saplings = 'DR' or 'LR', for field grown saplings a 'G' is added to the sample code and for potted saplings 'P'.

Regression coefficients: $ln(M) = a + bln(HD^2)$

		TOTAL BIOMASS						ABOVEGROUND BIOMASS					
Sample	Coefficient symbol	value	Variable SE	sig	MSE $ResSE^2$	CF $e^{(MSE/2)}$	$adjR^2$ (%)	value	Variable SE	sig.	MSE $ResSE^2$	CF $e^{(MSE/2)}$	$adjR^2$ (%)
						URBAN							
D	*a*	0.787	1.666	ns	0.170	1.09	69.96	-1.844	1.968	ns	0.237	1.126	68.57
	b	0.789	0.089	***				0.903	0.106	***			
DI	*a*	-1.760	2.751	ns	0.089	1.05	75.62	-3.315	2.953	ns	0.103	1.053	75.35
	b	0.941	0.146	***				1.003	0.157	***			
DR	*a*	2.694	1.481	ns	0.105	1.05	78.69	0.245	1.543	ns	0.114	1.058	81.78
	b	0.675	0.080	***				0.774	0.083	***			
DIG	*a*	1.555	3.492	ns	0.082	1.04	69.07	-0.754	3.973	ns	0.106	1.054	68.92
	b	0.762	0.187	***				0.864	0.213	**			
DIP	*a*	-5.834	4.654	ns	0.101	1.05	80.9	-6.160	5.080	ns	0.120	1.062	77.81
	b	1.159	0.246	**				1.157	0.269	*			
DRG	*a*	2.176	1.679	ns	0.106	1.05	86.73	0.328	1.740	ns	0.114	1.059	88.13
	b	0.698	0.090	***				0.770	0.094	***			
DRP	*a*	3.635	3.390	ns	0.107	1.05	54.21	-0.042	3.905	ns	0.141	1.073	58.84
	b	0.629	0.184	**				0.790	0.212	**			
L	*a*	1.905	1.611	ns	0.192	1.10	70.53	-2.351	1.490	ns	0.164	1.086	82.21
	b	0.743	0.092	***				0.951	0.085	***			
LI	*a*	0.445	2.442	ns	0.195	1.10	72.2	-0.631	2.100	ns	0.145	1.075	79.47
	b	0.827	0.135	***				0.865	0.116	***			
LR	*a*	8.264	2.981	***	0.145	1.08	21.72	3.205	2.261	ns	0.084	1.043	62.79
	b	0.365	0.175	ns				0.613	0.133	**			
LRG	*a*	6.014	0.135	*	0.000	1.00	99.94	2.718	0.123	*	0.000	1.000	99.97
	b	0.478	0.008	*				0.637	0.007	**			
LRP	*a*	11.4	4.223	*	0.131	1.07	-5.07	3.895	3.882	ns	0.111	1.057	37.19
	b	0.187	0.248	ns				0.574	0.228	*			
LIG	*a*	0.240	3.428	ns	0.214	1.11	64.45	0.136	3.154	ns	0.181	1.095	67.64
	b	0.834	0.191	**				0.821	0.175	**			
LIP	*a*	1.563	3.023	ns	0.129	1.07	87.5	-1.368	2.682	ns	0.102	1.052	92.5
	b	0.780	0.166	***				0.909	0.148	*			

at an advantage at planting (possibly due to increased nutrients and mychorrizal association in germination compost compared to bare root stock *Q. robur*), this has masked the increasing *RGR* of *Q. robur* (Figures 6.2 but see E.10 in appendix E).

Biomass allocation.

Allocation to $RWFt$ and $SWFt$ was greater in *Q. robur*, along with greater *SLA* (see chapter 5), while $LWFt$ and $BWFt$ were higher in *Q. ilex*. Despite greater $SWFt$ in the deciduous species this was not enough to increase $\overline{RGR}$ to rates higher than *Q. ilex* in this research, thus agreeing with (Struve et al. 2009). However the greater foliage and branch allocation in *Q. ilex* attributed to the greater overall biomass at final harvest in the evergreen saplings. Higher RWF allocation and lower LWF seem to be related not just to the deciduous leaf habit but also to reproductive strategy; the fire adapted oak, *Q. rubra* had the lowest $LWFt$ of four species measured and slowest *RGR* compared with two early and one late successional species, *Q. rubra* is a gap coloniser and the authors suggest allocation to roots helped its survival and ability to resprout after fire (Walters et al. 1993).

Nutrient availability tends to increase allocation to foliage and *AGB* although $LWFt$ also decreases with light (Poorter et al. 2012), this was not relevant to my research as all saplings were planted in open situations. Water availability can also effect allocation patterns with greater RWF allocated when water is scarce (Kuster et al. 2012), while stem and branch fractions can increase without water stress (Litton et al. 2007). Hamerlynck and Knapp (1996) found that red maple and Pine consistently gave greater allocation to roots when soil resources were low, whereas red oak increased RWF in response to decreased soil *N* but this increase was significant only when accompanied by increased water. Allocation patterns in this research show *Q. robur* did increase allocation to roots at nutrient limited sites but this was also linked to sites that were also water limited (L and B).

Urban influences.

Q. robur had reduced allocation to foliage at urban sites, although the urban site L was water limited, water stress was less obvious at D which may suggest other urban related factors

were reducing $LWFt$ at these sites. Other research shows a reduction in LWF for *Q. petrea* due to drought stress (Gieger and Thomas 2002) although it my also be a result of *Q. roburs* increased susceptibility to pollutants compared with *Q. ilex* (e.g. Calatayud et al. 2011). Further, Searle et al. (2012) found *Q. rubra* seedlings accumulated biomass at double the rate from rural and remote sites along a rural to urban gradient, however this research shows that despite higher CO_2 concentrations and warmer night time temperatures in urban situations, oak sapling growth is site specific and not always explained by strict 'urban' or 'rural' environments, at least within Plymouth where background NO_2 levels are relatively low compared to other cities (Anon 2008).

6.5 Conclusions and limitations.

At the sapling stage, increased allocation to foliage in the evergreen *Q. ilex* enables this species to acquire greater biomass and higher $\overline{RGR}$ over time than the deciduous *Q. robur* over the 30 month experimental period shown here. However, mean temperatures below 5°C put *Q. ilex* under stress and overall biomass is reduced due to lower allocation to foliage at this site in this species. In contrast, in the urban environment and at the drier rural site, Broadley, *Q. ilex* has greater total biomass than *Q. robur* and this was regardless of soil fertility (apart from at the urban site L where $RWFt$ was greater in pots than field grown saplings in this species). At the most favourable site, rural DT, both species were able to accumulate similar amounts of biomass over the experimental period.

The experimental period was short in the relative lifetime of a tree and growth strategies change with ontogeny. In general, young trees invest more carbon to foliage in an attempt to increase productivity (Poorter et al. 2012). The studies cited above analysed young seedlings from germination to 100 days and therefore are only able to infer *RGR* at seedling stage. My research gives a better idea of relative variability of *RGR* over the lifetime of a young tree after the initial seedling stage. However, relative growth rate was analysed using stem growth therefore further analyses of *RGR* in terms of *SLA* and ULR and *LAR* would be useful (see equation 6.7). Further, only 10-30% of *C* fixed during photosynthesis is using for wood production and further, overall biomass is poorly related to carbon assimilation as retention of past assimilation contained in

biomass (Litton et al. 2007). See also (Franco 1985) who suggested a model for tree growth based on living components of biomass.

$$RGR = ULRXSLAXLWF \tag{6.7}$$

6.5.1 Allometric Equations.

The search for simplicity in monitoring forest carbon stocks has led to a vast bank of information. From the theory of metabolic scaling (Enquist et al. 1998; Muller-Landau et al. 2006; Enquist, Allen, Brown, Gillooly, Kerkhoff, Niklas, Price and West 2007; Enquist, Tiffney and Niklas 2007) to the generalisation of equations for forest biomass at large (Zianis and Mencuccini 2004). Many authors have looked at the usefulness of additive parameters such as specific density (Muller-Landau 2004) and BEFs (Gracia and Sabate 2002).

Further analyses of the equations added here are necessary to elucidate the usefulness of site specific equations although it is need that population size at some of the more stressed locations will be unlikely to be useful. However, further modeling of the data is required. Specifically, adding density formulae and using the Akaike information criterion (AIC) where:

$$AIC = -2ln(L) + 2p \tag{6.8}$$

and L is the probability of the fitted model, p is the total number of parameters in the model and the best fit model reduces AIC (Chave et al. 2005).

Chapter 7

Mature Trees.

In order to aid the interpretation of ontogenetic[1] changes in photosynthesis and water use efficiency in *Q. ilex* and *Q. robur* a small canopy experiment was carried out and mean assimilation rates, photosynthetic efficiency and stomatal conductance were recorded in three mature trees of each species at one rural , urban and upland location within the Southwest. Results showed that although *Q. robur* had greater assimilation rates overall, this was only significant at the urban site, where low assimilation rates in *Q. ilex* coupled with closed stomata led to high water use efficiency but low carbon uptake at the urban site. Both species experienced a reduction in photosynthetic efficiency in sun leaves compared to shade. Performance index (PI ratio) was reduced in *Q. ilex* in upper canopy leaves due to the high light levels at the top of the canopy (7.08$\pm$ 0.08 upper sun leaves compared to 6.67 $\pm$ 0.08 lower sun leaves) and this effect was more prominent at the urban site. So, although *Q. ilex* had higher photosynthetic rates in March when the deciduous species was not in leaf, and recovered better from drought, evidenced by an increase in photosynthetic efficiency in the wetter June 2011 compared to June 2010, the down regulation of photosynthesis and stomatal control during higher temperatures interfered with carbon gain at the urban site for this species. However, milder winters and the ability of *Q. ilex* to withstand cold periods mean it is likely to become more common throughout the rural Southwest. Comments on ontogenetic differences are presented in the general discussion.

7.1 Introduction.

It is recognised that forests and forestry can have a substantial influence on the reduction of atmospheric carbon dioxide (CO_2) concentrations and are important for climate change mitiga-

[1] Relating to growth and developmental stages of an individual

tion (Anon 2003; Hegerl et al. 2007). In the UK, forest area has more than doubled over the past 80 years, and in 2007 was removing about 15 $MtCO_2$ per year (Read et al. 2009). However, Britains existing forests only cover around 12% of available land area, which is among the lowest of any country in Europe (Read et al. 2009). UK Forestry policy involves expansion of forest and short rotation forestry as well as the increased use of timber for construction (West and Gawith 2005). However, species choice is an important consideration and climate change mitigation using (a/re)fforestation has to take into account food production and national biodiversity targets (Morecroft et al. 2009).

Locally changing weather patterns mean that species productivity in relation to local environments also needs to be addressed. The potential of changing climate to interfere with a species range means the incorporation of non-native species for carbon sequestration may become more important in the future and the Forestry Commission conducts trials using non-native species (Willoughby et al. 2007). Range changes also mean that even without planting schemes, species composition in British woodlands is likely to change. Already there is an increasing presence of understory evergreen shrubs such as holly (Peterken 2001; Berger et al. 2007) and risk of invasion from non-native species is more likely. *Rhododendron ponticum* is considered invasive and reduces biodiversity, while Sycamore (*Acer pseudoplatanus*), a non-native gap species, has become more common in disturbed woodland (Epron and Dreyer 1993*a*).

There may be no advantage to either a deciduous or evergreen leaf habit (Royer et al. 2005; Dungan and Whitehead 2006). An evergreen tree may have a longer photosynthetic season, while the deciduous tree may make better use of its available growing season, or there may be trade-offs in relation to their environment: long lived leaves being an advantage in milder climates turn to a disadvantage when temperatures become very cold. To illustrate, *Q. robur* was hardier than *Q. ilex* with mean cold hardiness values in January of -56 and -27 °C for *Q. robur* and *Q. ilex*, respectively (Morin et al. 2007).

Further, the evergreen habit may be an adaptation for areas where soils are poor, an example of this was shown using *Quercus* spp.; the deciduous oak *Q. lobata* had higher photosynthesis and carbon sequestration potential than the evergreen *Q. agrifolia* when resources were plentiful but

lost this advantage when resources were scarce (Hollinger 1992).

Deciduous oaks are important forestry species throughout Europe. *Q. robur* is native to the UK and its natural range extends throughout Europe, SW Asia and North Africa (Kleinschmidt 1993). In the UK *Q. robur* is considered more drought sensitive compared to *Q. petrea*, and has suffered in areas that have become drier over the last 150 years (Epron and Dreyer 1993*a*). However, there is evidence that *Q. robur* is becoming more productive in upland areas of the Southwest UK with a more favourable climate (e.g., Wistmans wood, Dartmoor (Chavana-Bryant 2006)). Indeed, research in America has found differences in drought sensitivity between co-occurring deciduous oaks in the US and that tolerance to high temperatures determines species distribution at range limits (e.g. Hamerlynck and Knapp 1996). Non-native evergreen and deciduous oaks including *Q. ilex* and *Q. alba* have also been suggested as potentially useful for UK forestry if large climate changes occur (Read et al. 2009). *Q. ilex*, a schlerophyllus evergreen, is one of the dominant Mediterranean forest species and in its Mediterranean range tends to be restricted by water availability and winter temperatures (Ogaya and Penuelas 2007*a*). Its leaves may suffer from thermal stress above 35 °C (Gratani, Pesoli, Crescente, Aichner and Larcher 2000) but *Q. ilex* still grows on sites where the maximum air temperatures reach $40-50$ °C (Penuelas and Ogaya 2003). *Q.ilex* also shows large phenotypic plasticity throughout its range (Gimeno et al. 2009). *Q. ilex* is naturalised in Southern UK and is frequently found along the coast, in parks, and in urban areas throughout the Southwest. Although *Q. petrea* is the most widely found oak in the Southwest, *Q. robur* is still an important species in this area and can cope with water logging better than *Q. petrea* with increased productivity in this species found in lowland areas (Boisvenue and Running 2006). Given these considerations, and in order to aid evaluation of sapling data, we investigated net assimilation rates, water use efficiency, photosynthetic efficiency and leaf level carbon nitrogen patterns as well as specific leaf area (SLA) in mature trees of these two species at three contrasting sites.

1. General working hypotheses for mature trees:

    1.1. Are *Q. ilex* carbon assimilation rates and photosynthetic efficiency higher or lower than *Q. robur* for mature trees?

1.2. Are *Q. ilex* carbon assimilation rates and photosynthetic efficiency higher or lower than *Q. robur* in relation to urban, rural and upland environments?

1.3. At leaf level, do leaf state (sun and shade) and position in the canopy profile (lower middle and upper); affect assimilation rates and photosynthetic efficiency in either species?

1.4. Are leaf nitrogen (N_{leaf}) and leaf carbon (C_{leaf}) and SLA affected by any of the factors discussed and how does this relate to overall productivity for each species.

7.2 Methods.

7.2.1 Study sites.

Three locations, representative of upland, rural and urban areas, were chosen. The upland site was chosen as the highest site in SW England containing mature evergreen *Q. ilex*. This upland site is at Castle Drogo, [SX723901, 260 metres above sea level (m.a.s.l)], soils are thin, freely draining, acid and loamy soils over granite rock, typical upland soils of heath and moor (National Soil Resources Institute 2009). Both *Q. ilex* and *Q. robur* at this site are approximately 100 years old. The rural site is at Mount Edgcumbe, SE Cornwall. Here, *Q.ilex* is found at the 'top park', (SX457519, 60 m.a.s.l) and *Q. robur* is found at the Barrow car park area near Mount Edgcumbe House (SX451526). Soils at Mt Edgcumbe are freely draining, acid loams and can shallow near the top of the slope (National Soil Resources Institute 2009). Tree age at the rural site is between 100-150 years old, although these trees have possibly been pollarded in the past (Park Ranger pers. Comm.). Finally, the urban sites are Devonport Park (SX457550), Plymouth, for *Q. ilex* and Central Park (SX469560), Plymouth, for *Q. robur*. At 35 m.a.s.l. both sites are situated next to busy roads. Using historical evidence (e.g. Digimap 2009), tree age at both urban sites was estimated to be 120 years, again freely draining acid loams predominate but developed by the urban environment.

7.2.2 Climate Information.

In general climate in the Southwest UK is Oceanic with South-westerly dominating winds. Therefore this part of the UK experiences mild winters and warm wet summers with annual

Table 7.1: Climate statistics for the study sites (maximum, minimum, mean average temperate (MAT) and total rainfall). The first column provides both the distance between study site and the nearest meteorological station (m) from which the climatic information was obtained and their difference in height above sea-level.

m/m			$^{\circ}C$		mm
Site-station	Period	Max Temp	Min Temp	MAT	Total rainfall
Upland	Jun-10	19.7	9.8	14.6	39.1
3000/0	Mar-11	10.4	3	6.2	23.4
	Jun-11	17.1	9.1	12.7	74.9
	2010	11.8	5.2	8.4	946.2
	2004-2010	12.7	6.4	9.5	1101.3
Rural	Jun-10	22.4	8	15.6	26.2
3000/0	Mar-11	17.4	-1.7	8.5	36.4
	Jun-11	26.9	5.9	14.6	82.8
	2010	14.7	7.7	11.2	714.8
	2004-2010	14.3	8.2	11.3	962.6
Urban	Jun-10	23.5	9.6	15.9	37.5
1000/0	Mar-11	17.6	-0.1	8.8	29.4
	Jun-11	26.6	7.5	14.5	68.8
	2010	*	5.9	10.1	884.7
	2004-2010	*	8.2	11.1	989.4

Climatic data station information:
Rural, UK met office historical data for Mountbatten, Plymouth (50.35N, 04.12W)
Upland, Bridford, East Dartmoor Independent Weather station, (50.42N, 03.42W)
Urban, University of Plymouth Metnet archive, (50.22N, 04.08W).

temperatures averaging around 11 ° C, although the upland environment such as found on Dartmoor can experience average annual temperatures up to 2° lower (Jebson 2007). Annual rainfall averages for the South West region are around 900-1000mm in the lowland coastal areas with Princetown, 400masl having around twice as much (Jebson 2007). Climate records were obtained from the UK Met Office Southwest, and from Independent Weather stations at the University of Plymouth (situated at Drakes Circus, Plymouth) and Bridford (situated in East Dartmoor) (Table 7.1. See general methods section for complete climatic summary (chapter 2).

Nitrogen dioxide concentrations within Plymouth.

At the time of data collection the 2010 annual mean NO_2 concentrations for Tavistock road (situated next to Central park and *Q. robur* trees) were between 40 and 43 $\mu mol\ m^3$, close to and slightly above the target annual mean set out in the Environment Act 1995 of 40 $\mu mol\ m^3$ (Environmental Protection and Monitoring Unit 2009). However more recent data for the automatic monitoring site at Tavistock Road put annual means at 37.7 and 30.9 $\mu mol\ m^3$ in 2010 and 2011 respectively (Environmental Protection and Monitoring Unit 2012). The closest monitoring site in Alma road recorded annual mean of 36.1 and 33.1 $\mu mol\ m^3$ in 2010 and 2011 respectively (Environmental Protection and Monitoring Unit 2012). Background levels were 16.9 $\mu mol\ m^3$ in 2008, 36.5 $\mu mol\ m^3$ in 2010 and 29.6 $\mu mol\ m^3$ in 2011 (Environmental Protection and Monitoring Unit 2012). Therefore it can be assumed that both species are receiving some *N* deposition as both species are situated by the roadside at the urban sites.

7.2.3 Sampling Protocol.

During the initial data collection in June 2010, only two trees per species at each site were measured due to the time constraints imposed by using newly qualified canopy climbers and the need to complete measurement before 12pm when assimilation rates tend to drop. The following year it was possible to measure three *Q. ilex* per site during March 2011, before the deciduous oak came into leaf and the same three sampled again with three *Q. robur* in June 2011 at each site. The different levels of the canopy were accessed via ropes: Specifically using the double rope, 'Basic Canopy Access Procedure' (BCAP) to gain access to the canopy, followed by single rope technique to enable movement around the canopy.

Sampling was undertaken in the tree by trained volunteers from the Universal Canopy Exploration (UCE) group (see acknowledgments) and where access was difficult, branches cut and sent down to be measured at the base of the tree. All cut branches were placed in water to avoid water loss during sampling time and a small experiment showed no significant differences between cut and uncut branches for any of the parameters used ($p>0.05$). Parameters were measured on ten sun leaves and ten shade leaves from each canopy position (upper, middle and lower canopy). Lower canopy was accessed from the ground using a crook to bring branches closer if necessary, middle canopy was taken to be the area of the crown at its widest point ($\pm$ 2.5m) and the upper canopy the area as close to the top as possible but within 3 m of the top for difficult trees. For more open canopies where there was less distinction between sun and shade leaves, leaves were measured according to lightest (outer canopy) and shadiest (inner canopy) parts of the canopy.

All leaf level measurements were taken on 10 leaves per canopy level (LOWER/MIDDLE/UPPER) and leaf state (SUN/SHADE). Parameters measured were; Leaf level net photosynthetic rate (An $\mu mol\ m^2\ s^{-1}$) and stomatal conductance ($Gs\ mol\ m^2\ s^{-1}$) was measured using the LCi portable IRGA (ADC Bioscientific 2007). Intrinsic Water Use Efficiency (iWUE) measured as net photosynthetic rate (A_n) and stomatal conductance of water (Gs) ratio A_n/Gs ($\mu mol\ CO_2$ / $mol\ H_2O^{-1}$). The IRGA was used with a ADC light unit and irradiance set to 550-600 P.A.R. to enable comparison between sites and species at a consistent light level. Therefore any differences between sun and shade leaves are likely to be intrinsic due to the homogeneous light environment used.

Photosynthetic efficiency was measured using the plant efficiency analyser "Pocket PEA" (Hansatech Instruments Ltd 2010). The pocket PEA is a continuous excitation fluorometer that takes advantage of the red/far-red light energy re-emitted during the process of photosynthesis, and gives an indication of photosynthetic performance and plant health. Parameters used for analysis were; variable fluorescence over maximum fluorescence (F_v/F_m), a measure of maximum quantum efficiency of Photosystem II. F_v/F_m is a sensitive indication of plant photosynthetic performance, with 0.85 being the maximum obtained in a healthy plant, lower values are an

indication of stress (Hansatech Instruments Ltd 2007). Performance index (*PI*) developed by Strasser et al. (2000). *PI* incorporates three different stages of the fluorescence process to give an indication of plant vitality (Hermans et al. 2003). *PI* is more sensitive to stress than F_v/F_m and can exhibit a loss in performance during the energy transduction process in PSII (Clark et al. 2001). Both parameters are a measure of photosynthetic efficiency. Photosynthetic efficiency can be an indication of a plants reaction to drought and/or light stress. F_v/F_m itself is related to the potential for photochemical quenching or quantum yield of PSII (Strasser et al. 2000). Values less than 0.8 may indicate stress responses from the sample, and such reductions have been correlated with high light levels during the day (Cavender-Bares and Bazzaz 2004) and is often associated with photo-inhibition where leaves reduce their capacity for photochemical quenching as a protection from high light (Clark et al. 2000).

During June 2011 data collection, twenty leaves were taken from each factor level from each tree and taken to the lab in a cold storage box. Ten leaves from each factor level and tree were used to estimate specific leaf area *SLA*. this was done by first measuring fresh weight, then taking the leaf area using the LiCor Leaf Area Instrument. The leaves were then dried at 70° C for one week, or until two weights were the same. Dry weight was then taken and *SLA* and its inverse leaf mass per area *LMA* derived (*LMA* data not shown). The other half of the leaf samples were dried and ground to a fine powder using a burr grinder. The ground leaves were then weighed to 2 *mg* into small tin cups and then analysed in the Autoanalyser (see chapter 2 for more details on this procedure) for *N* and *C* concentrations (Leaf CN was measured on two separate occasions for seedlings and mature trees. The mature tree samples were kindly prepared and measured by Sarah Peake, Environmental Science graduate and volunteer).

Height and DBH were also taken of each tree sampled using a clinometer and DBH tape. For *Q. ilex* that had multiple stems DAG was taken. An estimate of biomass was taken using the general volume equation (HD^2) in cm^3 and this was used in a full factorial GLM using site and species as fixed factors. Mature tree biomass estimates are treated with caution due to differences in tree age between sites.

7.2.4 Analysis of data.

Statistical tests were performed on pooled leaf data (i.e. ten leaves for each leaf state and canopy position averaged) on three trees per species; this was to avoid pseudo-replication within the canopy layers. Data was analysed with general mixed linear models (GMLM) using R (R 2009). Model one looked for differences between sites and species for each parameter, models two and three analysed differences between dates for each species separately and model four analysed species (SP), site (S: Urban, Rural, Upland) leaf state (STATE: shade, Sun) and leaf position (LP: Lower, Middle, Upper) in a full factoral design just using the June 2011 data to simplify interpretation of the results.

All model parameters met conditions for normality (equal variances using Levenes test, p>0.05). Values are presented with means and std deviations when shown as descriptive statistics and means $\pm$ 95% confidence intervals when used in graphs to aid interpretation of significant differences.

7.3 Results.

7.3.1 Differences between species and sites.

Water use efficiency and photosynthetic parameters

Mean A_n was higher in *Q. robur* than *Q. ilex* at all sites, mean A_n was significantly lower at the urban site in *Q. ilex* than at the other sites for this species while also significantly lower than *Q. robur* at this site (Tables 7.2 and 7.5). *Q. ilex Gs* was more variable than in *Q. robur* and was significantly lower at the urban site than all other factor levels (Table 7.2). $iWUE$ was lower in *Q. ilex* than *Q. robur* and the combination of increased A_n and moderate *Gs* in *Q. robur* has lowered its $iWUE$ at the urban site where in fact both species have lower $iWUE$ (Tables 7.2 and 7.5). F_v/F_m and *PI* were lower in *Q. robur* than *Q. ilex* at the upland site, where mean *PI* was nearly twice as high in *Q. ilex* (Tables 7.2 and 7.5).

leaf traits.

SLA was significantly higher in *Q. robur* than *Q. ilex*, the same was true for N_{leaf}, however, for C_{leaf} the opposite was true. N_{leaf} was higher at the urban site than at the other sites in *Q. robur* and C_{leaf} was significantly lower at the urban site in both species (Table 7.3).

7.3.2 Differences between dates.

Water use efficiency and photosynthetic parameters.

There were significant differences between dates for both species in $iWUE$, however although both species also showed an interaction between date and site, there was no significant effect of site on *Q. robur* $iWUE$ in mature trees (Table 7.4 for ANOVA results and appendix H for graphs). $iWUE$ was significantly higher in June 2011 than June 2010 for both upland and rural sites but not the urban site in *Q. robur*, whereas in *Q. ilex*, $iWUE$ was significantly higher in March 2011 and June 2011 than the previous June except at the urban site where June 2011 is lower than the previous March (Table 7.4 for ANOVA results and appendix H for graphs). This corresponds with lower monthly rainfall in June 2010 than in June 2011.

There was an effect of all factors and interaction in both species for mean A_n (Table 7.4). Closer inspection of the data showed that in *Q. ilex* A_n was higher in March (when *Q. robur* was not in leaf) than June (both dates). For *Q. robur*, there was a slight increase in mean A_n rates in June 2011 at the uplands site although the opposite was true at the urban site (appendix H Figure H.3). However, in *Q. ilex* photosynthetic efficiency was low in March compared to June dates (F_v/F_m mean values 0.77 ± 0.04 in March compared to 0.82 ± 0.03 in June and PI mean values of 6.1 ± 1.88 and 9.2 ± 2.68 respectively). There was also an increase in *PI* values between June 2010 and 2011 at the urban and uplands sites for *Q. robur* and for all sites in *Q. ilex* (See appendix H for figures).

Table 7.2: Descriptive statistics for mature tree physiological parameters; mean assimilation rates (A_n $\mu mol\ m^{-2}\ s^{-1}$), intrinsic water use efficiency (iWUE as A_n / Gs $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ / $mol\ H_2O\ s^{-}1$), stomatal conductance ($mmol\ m^{-2}\ s^{-1}$) (means and standard deviations, capital letters show species differences between sites and lower case letters show significant differences between species).

	No.	F_v/F_m	StD		PI	StD		Gs	StD		$iWUE$	StD		A_n	StD	
							Q. ilex									
UPLAND	18	0.82	0.016	Aa	9.44	2.26	Aa	0.18	0.146	Aa	55.0	30.6	Aa	5.91	1.86	Aac
RURAL	18	0.82	0.015	Aa	8.57	1.94	Ac	0.32	0.560	Ac	49.9	36.8	Ab	4.61	2.39	Aab
URBAN	18	0.82	0.027	Aa	9.56	3.59	Aa	0.08	0.030	Bc	44.0	14.0	Ab	3.12	1.13	Bb
							Q. robur									
UPLAND	18	0.79	0.043	Ba	4.21	1.39	Bb	0.12	0.069	Aac	64.9	24.2	Aab	6.17	2.44	Aac
RURAL	18	0.81	0.035	Aa	7.10	2.34	Abc	0.10	0.102	Abc	75.2	30.9	Aac	5.55	3.48	Aac
URBAN	18	0.83	0.012	Aa	8.07	1.99	Aac	0.12	0.053	Aac	42.6	16.5	Bab	5.11	2.45	Ac

Table 7.3: Mature tree leaf traits: specific leaf area (SLA) $cm^2\ g^{-1}$, leaf nitrogen N_{leaf} %, and leaf carbon C_{leaf} %. Sample size (N), means, standard deviations (StD) and significant differences between sites (group) for each species (capital letters) and between sites (lower case letters)

Descriptive statistics for canopy leaf traits

Leaf trait	No.	*SLA*	StD	group	N_{leaf}	StD	group	C_{leaf}	StD	group
					Q. ilex					
UPLAND	18	6.82	1.605	Aa	1.272	0.197	Aa	47.999	0.968	ABb
RURAL	18	8.38	1.553	Ab	1.363	0.278	Ac	48.133	0.982	ABb
URBAN	18	7.53	1.855	Ac	1.361	0.279	Ae	47.220	0.803	Ba
					Q. robur					
UPLAND	18	16.84	6.681	Ad	2.128	0.406	Ab	47.336	0.930	Aa
RURAL	18	18.34	5.473	Ad	2.221	0.389	Ad	47.245	0.777	Aa
URBAN	18	16.23	4.467	Ad	2.468	0.345	Af	46.565	0.779	Aa

Table 7.4: Differences in physiological parameters between dates (June 2010, March 2011 (*Q. ilex* only) and Jun 2011). Differences in intrinsic water use efficiency ($iWUE$) A/Gs $\mu mol\ s^{-1}\ m^{-2}$ / $mol\ H_2O^{-1}$, net assimilation rate (A_n) $\mu mol\ s^{-1}\ m^{-2}$, Variable fluorescence over maximum fluorescence ratio F_v/F_m and performance index PI parameters shown for sites and dates for **A** *Q. ilex* and **B** *Q. robur*.

A) *Q. ilex*

ANOVA table: Canopy physiological variables

		$iWUE$		GS		An		F_vF_m		PI	
	Df	F	sig	F	sig	F	sig	F	sig	F	sig
DATE	2	6.54	**	0.77	ns	27.62	***	35.70	***	38.01	***
SITE	2	2.12	ns	5.96	**	7.39	***	1.57	ns	1.63	ns
DATE:SITE	4	2.77	*	1.88	ns	2.50	*	1.28	ns	1.10	ns
Residuals	135										

B) *Q. robur*

		$iWUE$		F_v/F_m		PI	
	Df	F	sig	F	sig	F	sig
DATE	1	8.31	**	1.23	ns	10.87	**
SITE	2	4.62	*	5.53	**	28.95	***
DATE:SITE	2	8.37	***	6.69	**	8.89	***
Residuals	84						

7.3.3 Differences in sun and shade leaves and position in the canopy.

Water-use efficiency and photosynthetic parameters.

Mean A_n was significantly higher in sun leaves than shade leave for both species (Figure 7.1) and although there was no effect of LP on this parameter, a complex interaction existed where *Q. robur* mean A_n was significantly lower in the upper canopy sun leaves at the rural site (2.9 $\pm$ 0.12 SUN; 3.7 $\pm$ 0.49 SHADE) whereas the opposite is true for *Q. ilex* (Table 7.5, Figure 7.1). Mean *Gs* and $iWUE$ were not affected by LP or STATE but $iWUE$ did share an interaction between Species, LP and site where *Q. robur* had significantly greater $iWUE$ in the lower canopy at the upland site compared with *Q. ilex* (22.6 $\pm$ 12.88 I; 78.7 $\pm$ 6.12 R) (Figure 7.3). F_v/F_m and *PI* were significantly lower in sun than shade leaves for both species (SUN 0.81 $\pm$ 0.02; SHADE 0.83 $\pm$ 0.01 for *Q. ilex* and SUN 0.79 $\pm$ 0.04; SHADE 0.83 $\pm$ 0.01 for *Q. robur*) (Table 7.5 and Figure 7.4). F_v/F_m was reduced in upper canopy in *Q. ilex* leaves although this effect was not significant 7.4.

leaf traits.

SLA was only significantly affected by species and leaf state and there were significant differences at all levels of the interaction between species and state. Sun leaves are significantly lower *SLA* than shade in both species (SUN: 67.69 $\pm$ 19.76; SHADE: 83.77 $\pm$ 10.50 in *Q. ilex* compared to SUN 142.69 $\pm$ 50.30; SHADE: 200 $\pm$ 45.87 in *Q. robur*) (see Table 7.6 and Figure 7.6 different letters show significant differences using Tukeys HSD). There was an effect of leaf state (STATE) on C_{leaf}, although on average SUN leaves had 0.4 % higher C_{leaf}, however in *Q. robur* mean difference was 0.6 % higher in SUN leaves (Figure 7.8). In contrast N_{leaf} had an effect of leaf state, while leaf position interacted with species and site (Table 7.5, Figure 7.8). N_{leaf} is significantly lower in sun than shade leaves (SUN 1.4 $\pm$ 0.99; SHADE 1.2 $\pm$ 0.96 *Q. ilex* and SUN 2.4 $\pm$ 0.69; SHADE 2.18 $\pm$ 0.93 *Q. robur*) and this distinction is more prominent in the lower canopy in *Q. ilex*. *Q. robur* upper canopy leaves had higher N_{leaf} at the urban and rural sites (Figure 7.8).

Table 7.5: Leaf physiological parameters, differences between sun and shade (STATE), position in the canopy (LP) and species.

ANOVA: Leaf physiology variables

Response:		*Gs*		*iWUE*		A_n		F_v/F_m		*PI*	
	Df	F	sig	F	sig	F	sig	F	sig	F	sig
SPECIES	1	2.61	ns	5.63	*	6.07	*	7.24	**	41.4	***
SITE	2	1.91	ns	6.49	**	6.61	**	7.48	***	7.30	**
STATE	1	4.53	*	0.15	ns	15.8	***	54.3	***	0.08	ns
LP	2	0.03	ns	0.16	ns	1.34	ns			2.05	ns
SPECIES:SITE	2			2.67	ns			9.12	***	8.65	***
SPECIES:LP	2			1.94	ns			9.82	**		
SITE:LP	4			2.54	*			2.91	ns		
SPECIES:SITE:LP	4			4.25	**			2.90	ns		
SPECIES:SITE:STATE:LP	4				ns				ns	2.88	*
Residuals	101			89.00		101		96		72	

Table 7.6: Effects of Leaf state (SUN SHADE, species and interactions. No effect of canopy position therefore data not shown (see glossary for parameter units).

ANOVA:Morphological leaf trait SLA

Response:		*SLA*		
	Df	F	p	sig
SPECIES	1	192	0.00	***
STATE	1	28.3	0.00	***
SPECIES:STATE	1	8.94	0.003	**
Residuals	104			

Table 7.7: Canopy leaf N and C. Leaf state, canopy position and site effects for each species.

ANOVA table

Leaf trait		N_{leaf}		C_{leaf}	
	Df	F	P	F	P
SPECIES	1	883.85	0.00***	18.23	0.00***
SITE	4	20.18	0.00***	10.79	0.00***
STATE	1	10.11	0.00***	58.18	0.00***
SPECIES:LP	1	4.31	0.04*		
SPECIES:SITE:LP	4	3.19	0.02*		
Residuals	89				

Comparison of estimated biomass in *Q. robur* and *Q. ilex*.

Biomass was significantly higher in *Q. robur* than *Q. ilex* ($F_{(1,13)}$ = 7.67, p<0.05) and an interaction between species and site ($F_{(2,13)}$ = 6.18, p<0.05) showed that *Q. ilex* biomass was higher at the upland site than *Q. robur* (829.3 $\pm$ 476.75 m^3 compared to 567.17 $\pm$ 328.45 m^3).

Table 7.8: Estimated biomass shown as volume estimate in m^3 (means and standard deviations shown).

Descriptive Statistics for Canopy Biomass

SPECIES	SITE	No.	mean HD^2	StDev
Q. ilex	RURAL	3	548	556
	UPLAND	3	829	477
	URBAN	3	503	50.1
Q. robur	RURAL	3	1790	328
	UPLAND	3	567	329
	URBAN	3	991	192

7.4 Discussion.

7.4.1 Differences between species and environment.

Deciduous oaks have higher maximum assimilation rates than their evergreen counterparts. However, the evergreen *Q. ilex* achieves maximum photosynthetic rates during spring and autumn when air temperatures are mild and water not limiting (Baldocchi et al. 2010; Vaz et al. 2010). This is also true during the winter months when deciduous oaks are not in leaf. In fact, my research shows that *Q. ilex* winter assimilation rates can increase to rates comparable with *Q. robur* summer rates. In contrast, *Q. robur* has been shown to increase assimilation rates quickly with increasing light (Baldocchi et al. 2010), however, increasing temperatures also allow *Q. robur* to increase assimilation rates irrespective of increasing light (as evidenced here by using the light unit). *Q. robur* has to make efficient use of the photosynthetic season and will increase its assimilation rates during higher temperatures even when short term drought occurs (Drobyshev et al. 2008). This'anisohydric' can interfere with its water saving strategy of stomatal control and can leave it vulnerable to further drought episodes (Cater and Batic 2006; Sade et al. 2012).

So, evergreen broadleaved trees show a restrained response to temperature increases compared with deciduous trees (Way and Oren 2010), which for *Q. ilex* may be intrinsic adaption to drought stress in the Mediterranean environment. My research showed *Q. robur* had greater $iWUE$ than *Q. ilex* and in general it maintained higher photosynthetic rates despite lowered *Gs*, this has been discussed by previous authors for *Q. robur* (e.g., Epron and Dreyer 1993*a*;

Kazda et al. 2000). However, despite increasing assimilation rates giving the deciduous oak an advantage over the summer months, the evergreen oaks tighter stomatal control, means it can recover from drought stress more readily (Chaves et al. 2002).

Reduced $iWUE$ at the urban site may be a combination of restricted rooting depth and water availability, a direct result of the urban environment, controlling stomatal conductance and interacting with the effects of particulate pollution at that site. Research suggests benefits for planting evergreen oaks in the urban environment; in general, broadleaved species with rough surfaces are better at collecting particles, and indeed *Q. robur* was able to absorb significant amounts of pollutant before showing sign of stress (Beckett et al. 2000). However, in another study, the evergreen *Q. ilex* was better at reducing pollution and air temperatures in Rome than the deciduous *Q. pubescens*, the leaves not only absorbing pollutants but also reducing air temperatures and humidity in the City (Gratani, Crescente and Petruzzi 2000). There were also benefits for being photosynthetically active and maintaining a canopy all year round (Gratani and Varone 2006) although of the mature oaks in my research, *Q. ilex* trees were showing signs of stress via stomatal control (but see also Ugolini et al. 2012). Despite this the urban environment does not seem to have affected photosynthetic efficiency in either species despite the stomatal control and lowered water use efficiency of both species growing in Plymouth City Centre.

In terms of photosynthetic efficiency, *Q. ilex* also seems better able to recover from stress than *Q. robur* and *PI* remains lower in *Q. robur* at all sites while F_v/F_m, is lower in *Q. robur* than *Q. ilex* at the upland site (Castle Drogo, East Dartmoor). This suggests that during the summer months when trade-offs between stress responses and assimilation rates occur in *Q. ilex* electron transport and photochemical quenching remain efficient. However, winter photosynthetic efficiency in evergreen broadleaves show signs of photo-inhibition in the Mediterranean environment (Martinez-Ferri et al. 2004) as here evidenced by the reduced F_v/F_m and *PI* values during March for *Q. ilex*. In contrast *Q. robur* showed signs of stress in the upland environment. Indeed this oak is showing growth decline in many parts of its European range and this decline has been directly associated with regional climate change (e.g. Drobyshev et al. 2008).

7.4.2 Differences between sun and shade leaves and their position in the canopy.

Water use efficiency and photosynthetic parameters

Assimilation rates of sun leaves tend to be higher than shade leaves in deciduous tree species, as was found in *Q. crispula*, a deciduous oak found in the Far East, which had assimilation rates of 13.4 and 6.7 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ in sun and shade leaves respectively comparable to those found here (Koike et al. 2004). However, the same study found no difference between sun and shade in the upper canopy (Koike et al. 2004). In my study, *Q. robur* experienced reduced photosynthesis in the upper canopy despite no reduction in photosynthetic efficiency at this height (e.g., by limiting light levels by using a light unit), an indication that although there may be an intrinsic response to high light and temperatures in assimilation rate at the upper canopy any reduction in photochemical quenching at this height is likely to be reversible for this species. Other research has shown that upper canopy leaves from a deciduous red oak (*Q. rubra*) and *Q. ilex* experienced reduced F_v/F_m in upper canopy sun leaves compared to the lower canopy (Manes et al. 1997; Cavender-Bares and Bazzaz 2004).

In contrast, in *Q. ilex*, there is an indication of reduced photosynthetic efficiency, implying photo-inhibition in the upper canopy, although the signature is small which again may be due to the use of the light unit where light levels remained at saturation levels but not higher, thus not inducing photo-protective down regulation of the photosynthetic apparatus and indication that reversible photoinhibition is more evident at rural and upland sites but that more permanent damage has occurred at the urban site. Molchanov (2005) found that for shade and sun leaves in the upper canopy, photosynthetic efficiency depended on solar radiation, water availability and air temperatures. The reduced performance and vitality shown by a reduction in these parameters during March for *Q. ilex* indicate that this species is less tolerant of high light during lower temperatures and indeed it is has been suggested that *Q. ilex* experiences photo-inhibition during periods of cold stress (Ogaya and Penuelas 2003; Valladares et al. 2005) This has also been shown in other evergreen broadleaved trees such as *Ilex aquifolium* growing in a Mediterranean environment (Valladares et al. 2005) (see appendix H for data showing differences between dates).

Leaf level C and N and SLA.

N_{leaf}, measured on a mass basis, was lower in sun leaves than shade in *Q. ilex* and this trend was more obvious in the upper canopy and in the urban environment. In *Q. robur* only the upland site had lower N_{leaf} in sun versus shade leaves. In fact, for *Q. robur*, without taking into account leaf state and position in the canopy, the urban environment had higher N_{leaf} than the other two sites. Although N_{leaf} measured on a mass basis seems to have less predictive power for assimilation rates than area based measurements, the inverse of *SLA*, *LMA* is a good predictor of net assimilation. Therefore *SLA* decreases with increasing irradiance. Upper canopy leaves had greatest N_{area} because of their high *LMA* in *Q. robur* although N_{mass} was lower, the high N_{area} reflected high assimilation rates (10.7+ $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) as N_{leaf} is distributed through the canopy in order to maximise photosynthesis (Kazda et al. 2000).

7.4.3 Overall biomass estimates and conclusions.

Although the biomass estimates for mature trees in this study are based on volume estimates and are only comparable between species at each site due to age related differences, the results indicate that *Q. robur* is better at accumulating carbon over time even in the urban environment. This suggests that perhaps increased *N* deposition to the leaf enables faster utilisation and consequently greater productivity for the deciduous oaks. The evergreen may not be able to utilise the *N* deposited on the waxy cuticle as efficiently, however *Q. ilex* leaves have been shown to be efficient bioaccumulators of *N* in urban areas of Italy (Alfani et al. 2000). However the *Q. robur* trees at Castle Drogo are under stress which is indicated by reduced photosynthetic efficiency and relative assimilation rates in this species at this site. Mature oaks growing in the oceanic environment within the Southwest, are subjected to increases in pathogen attacks such as the Honey fungus and various *Phytophorum* species, as well as increased defoliation from insects and caterpillars. Therefore, summer defoliation, combined with poor soils and soil water availability is perhaps the reason for reduced growth in this species at the upland site. Indeed, at 280m above sea level, the Castle Drogo trees experience extended frosts, which have caused cavitation and loss of limbs from many of the mature *Q. ilex* there (pers. obs). Efficiency is lower during the winter months despite milder temperatures increasing assimilation rates.

However, the competitive ability of *Q. ilex* to persist on such soils in its Mediterranean range along with its resilience to drought and temperature stress may give it an ecological advantage in future climate scenarios in the UK. Indeed, *Q. ilex* has already become a "pest" species for wildlife managers along the south coast of Britain but may become more prominent within the next 100 years.

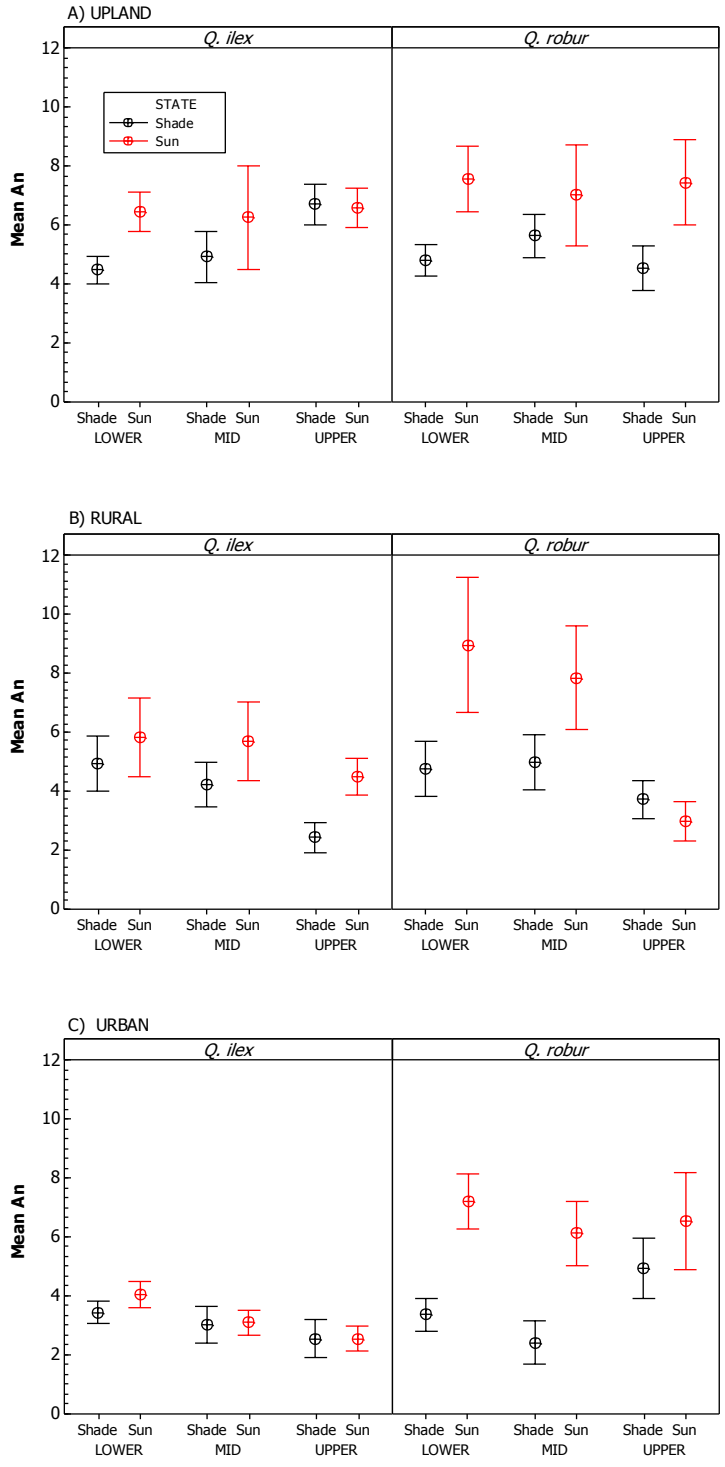


Figure 7.1: Differences in carbon assimilation rate (A_n) (μ *mols* s^{-1} m^{-2}) for sun and shade leaves in lower, middle and upper canopy for each site; A upland, B rural and C urban (means $\pm$ 95 % CI).

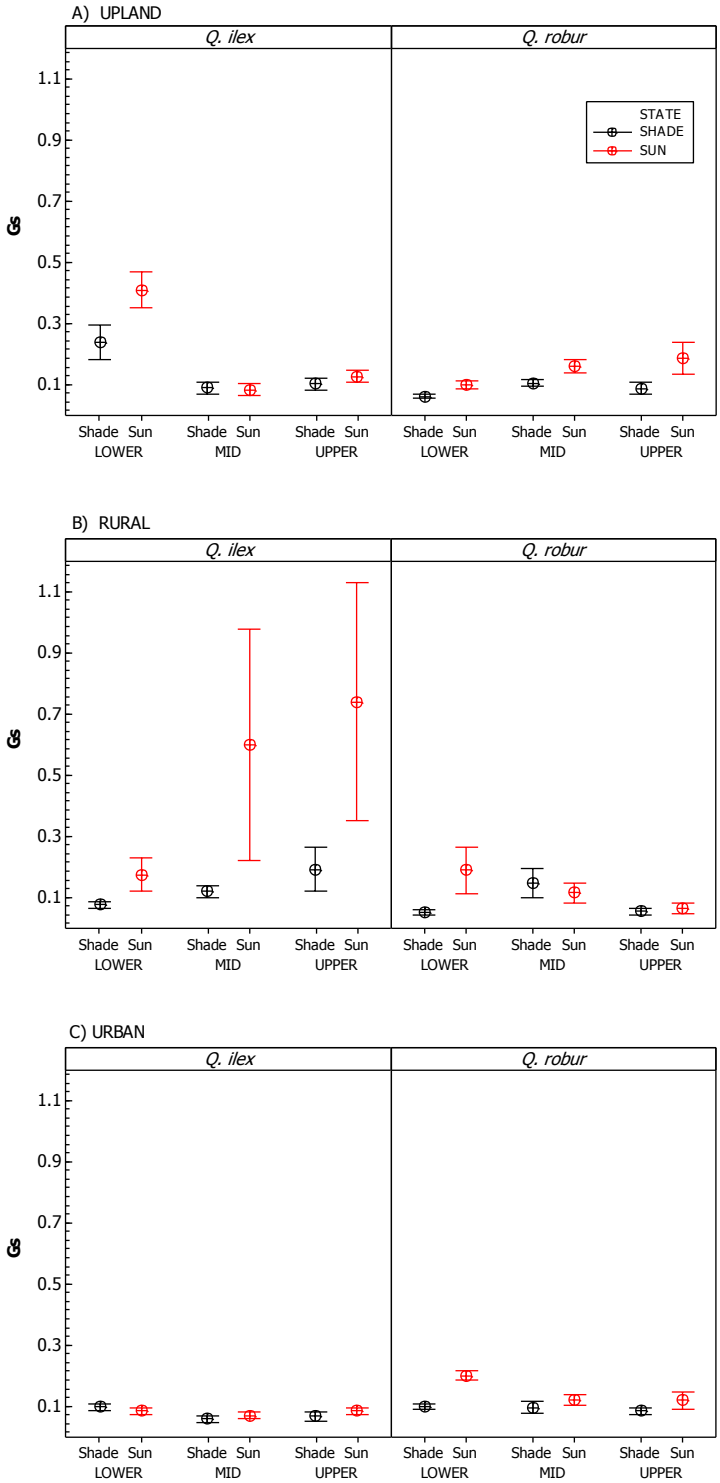


Figure 7.2: Differences in canopy stomatal conductance (Gs) ($m\ mols\ s^{-1}m^{-2}$) for sun and shade leaves in lower, middle and upper canopy for each site; A upland, B rural and C urban (means $\pm$ 95 % CI).

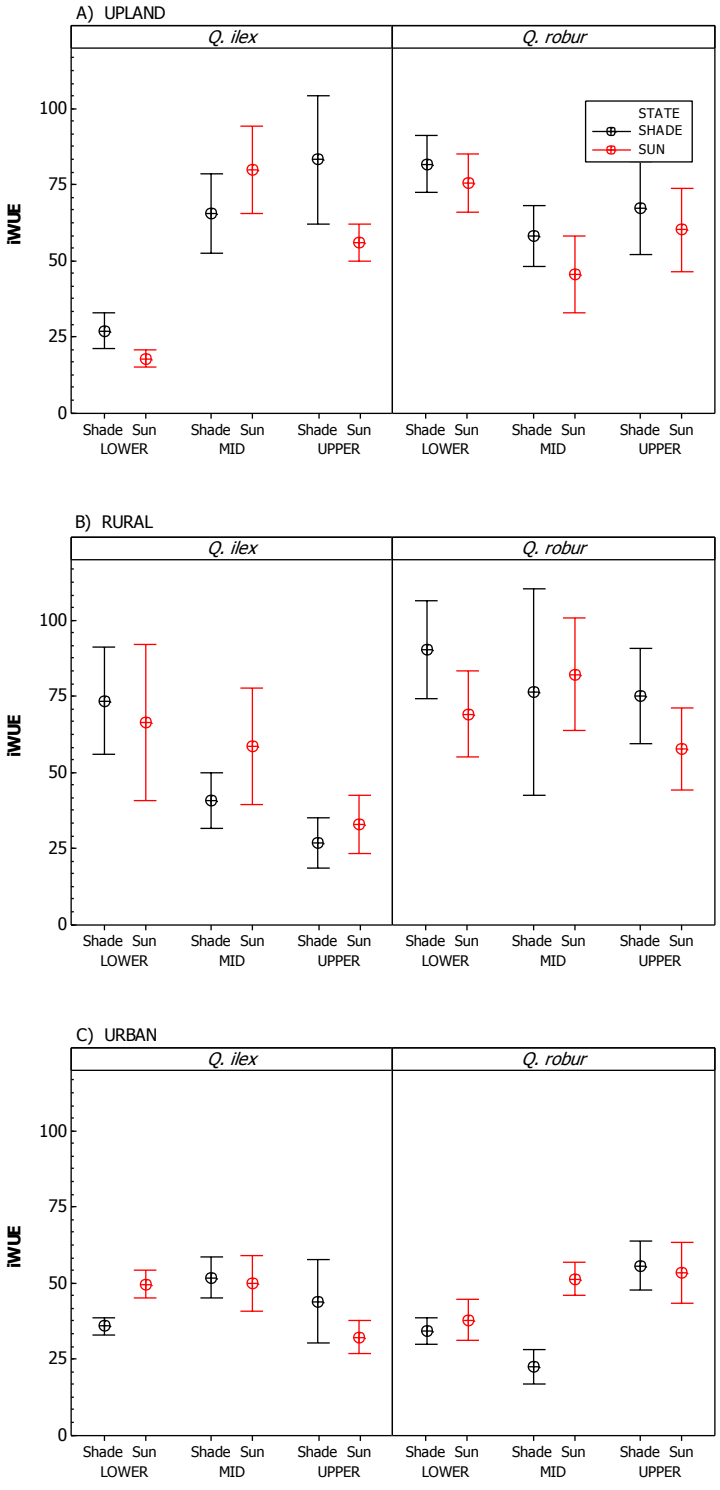


Figure 7.3: Differences in canopy intrinsic water use efficiency (iWUE) (measured as Mean assimilation rate and stomatal conductance ratio) for sun and shade leaves in lower, middle and upper canopy for each site; A upland, B rural and C urban (means ± 95 % CI).

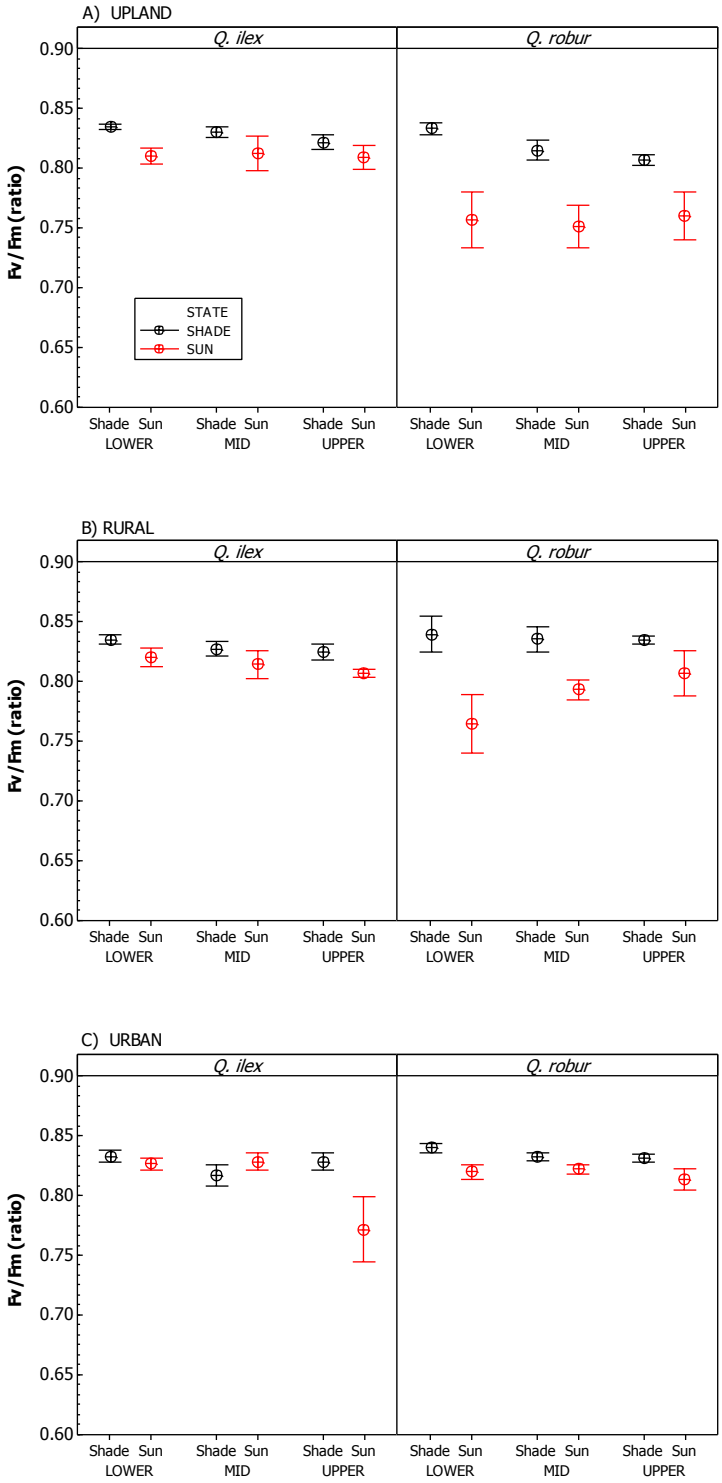


Figure 7.4: Differences in canopy F_v/F_m as a measure of maximum photochemical quenching of electrons in photosystem II, for sun and shade leaves in lower, middle and upper canopy for each site; A upland, B rural and C urban (means $\pm$ 95 % CI).

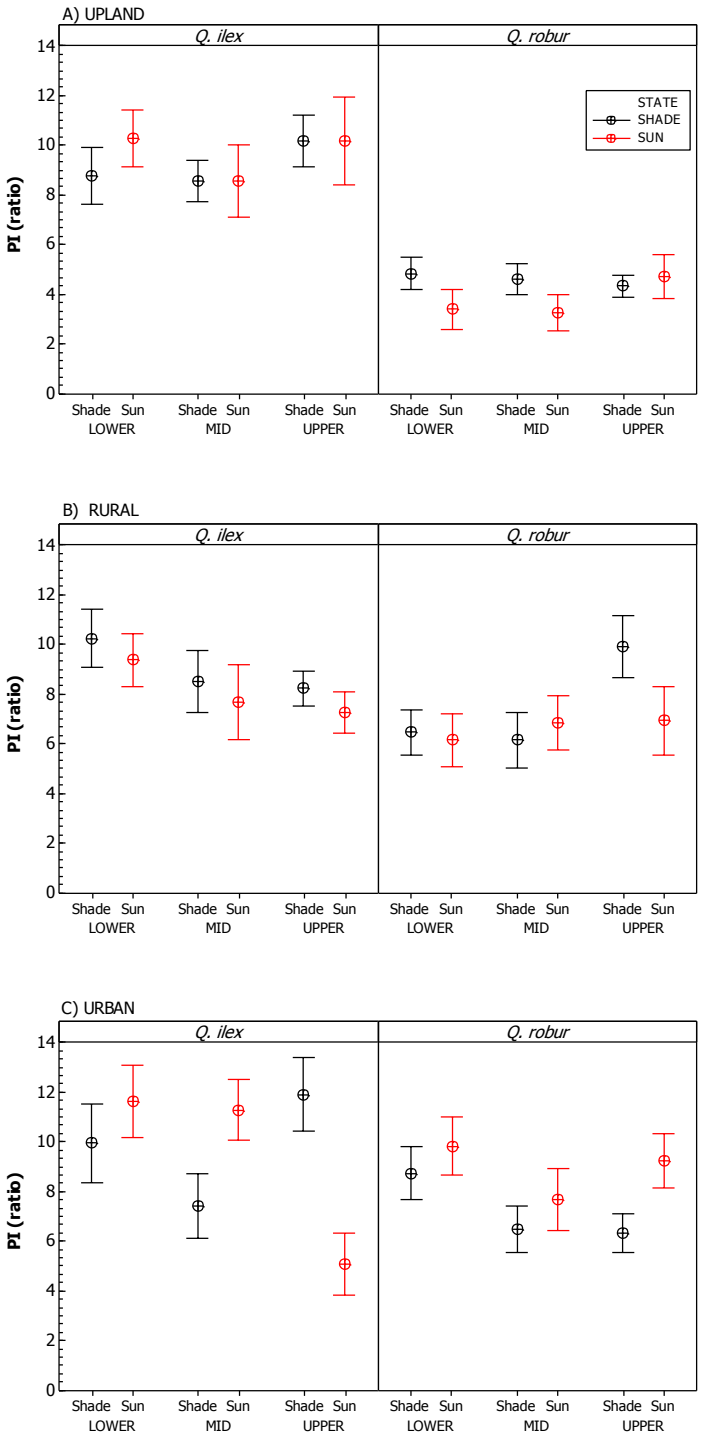


Figure 7.5: Differences in canopy performance index PI (a measure of sapling vitality and consisting of three different measurements of chlorophyll fluorescence emissions taken over the fluorescence rise see Strasser et al. (2000) for full explanation of PI) for sun and shade leaves in lower, middle and upper canopy for each site; A upland, B rural and C urban (means $\pm$ 95 % CI).

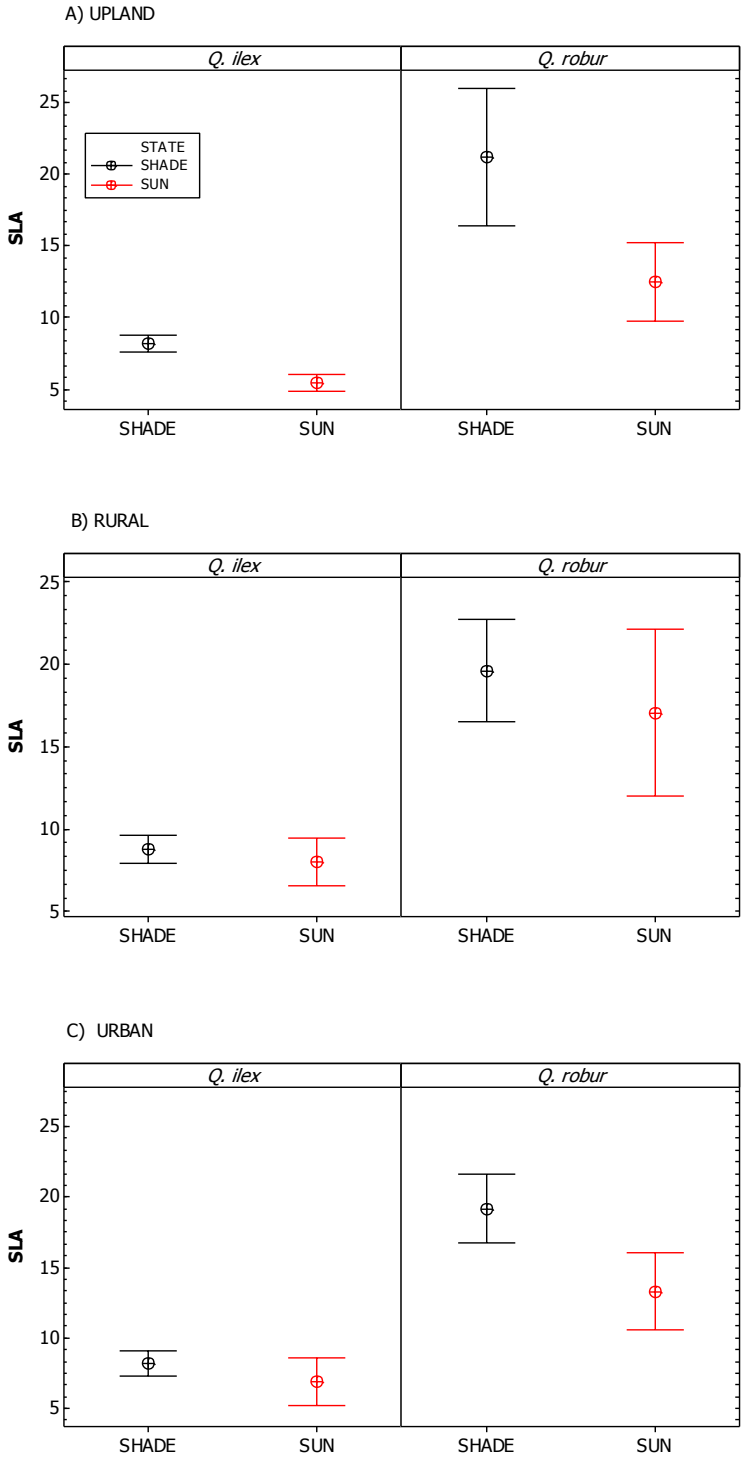


Figure 7.6: Differences in canopy specific leaf area (SLA), effect of Species and leaf state for each site; A upland, B rural and C urban (means $\pm$ 95 % CI). (means $\pm$ 95 % CI).

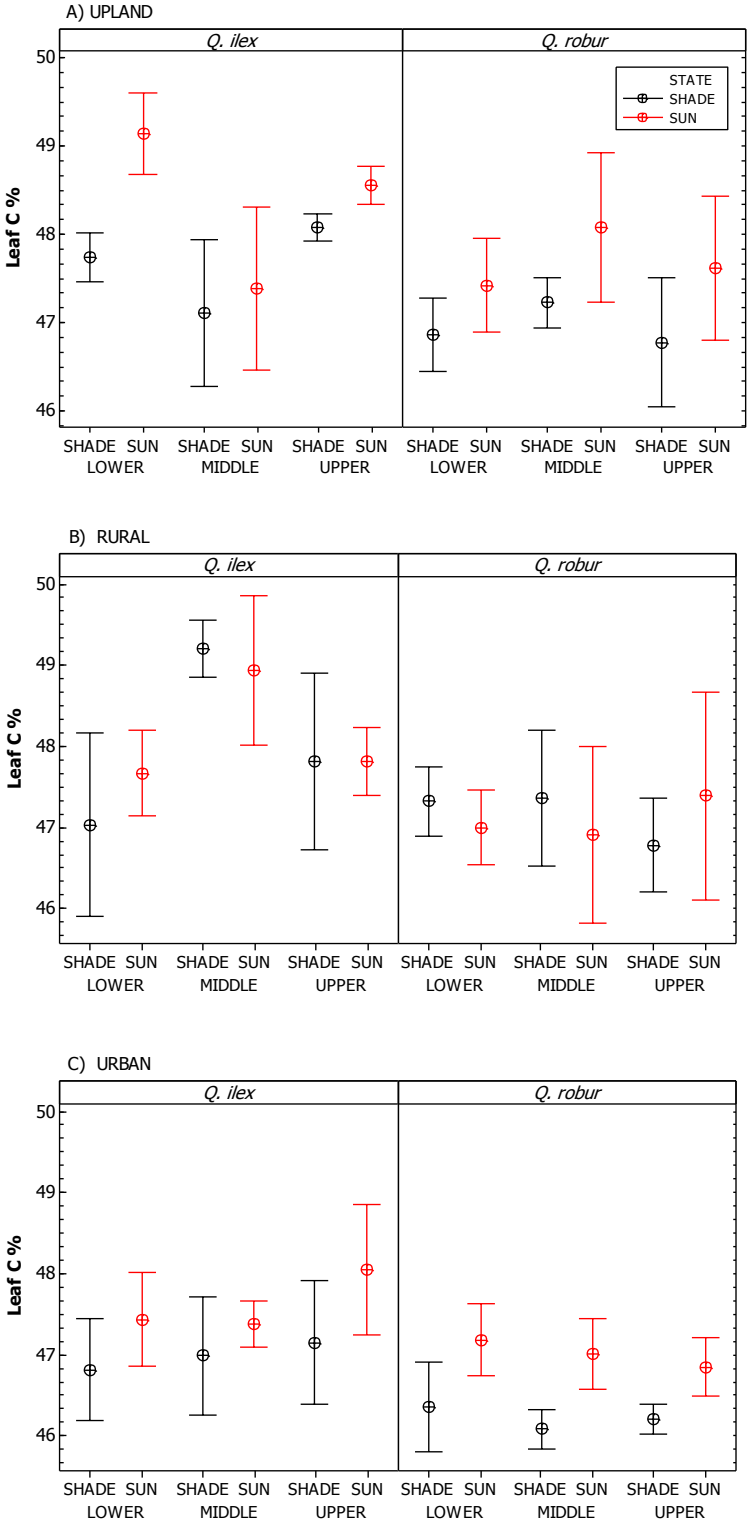


Figure 7.7: Differences in canopy C_{leaf} for sun and shade leaves in lower, middle and upper canopy for each site; A upland, B rural and C urban (means $\pm$ 95 % CI).

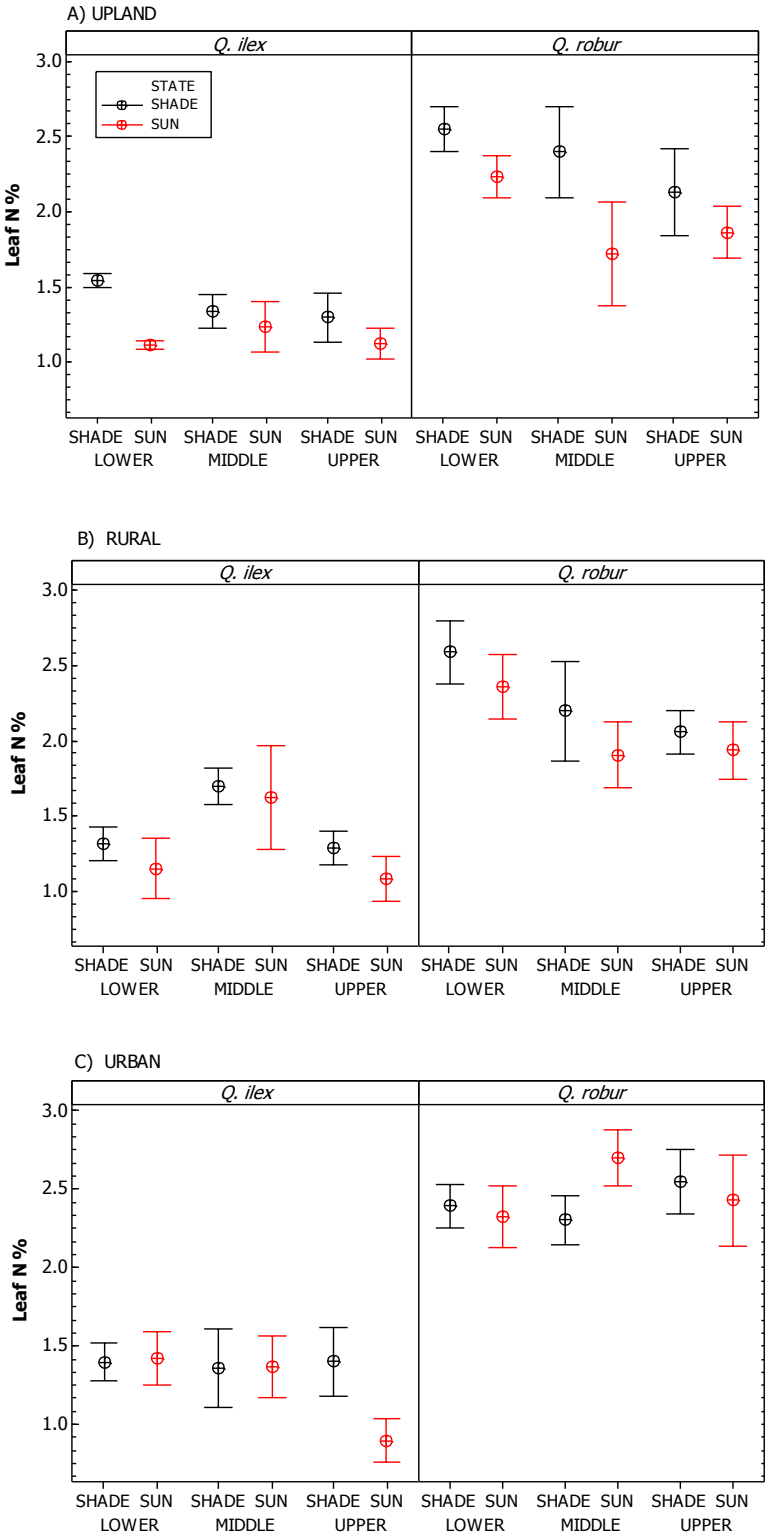


Figure 7.8: Differences in canopy N_{leaf} for sun and shade leaves in lower, middle and upper canopy for each site; A upland, B rural and C urban (means $\pm$ 95 % CI).

Chapter 8

General Discussion.

This research set out to investigate photosynthetic efficiency and carbon sequestration potential of the deciduous Pedunculate oak (*Q. robur* L.) and the evergreen Holm oak (*Q. ilex* subsp. *ilex* L.) in relation to their environment within the temperate, 'oceanic' climate of the Southwest. The former species, a UK native and common in lowland Southwest England, is drought sensitive (Epron and Dreyer 1993*a*) but copes well with waterlogged soils (Parelle et al. 2007). In comparison the evergreen has a natural distribution within the sub humid Mediterranean regions, western and central Mediterranean and North Africa, however it can also be found in temperate humid climates but only colonizes more arid areas where temperatures are much colder (Barbero et al. 1992). This suggests that the oceanic, temperate climate of the southwest could become more favourable towards *Q.ilex* with future climate scenarios; however, in this type of environment competition from deciduous oaks is also more likely (Barbero et al. 1992). Further, *Q. ilex* has become more common in South and Southeast UK, and is known to regenerate freely in some areas along the South coast (Plantlife 2010). *Q. ilex* has also been widely planted (historically) within the Southwest and has become a dominant species in some woodlands such as Mt Edgcumbe in SE Cornwall, while also found as a planted tree throughout Plymouth city centre (pers. obs.).

Alternatively, increased productivity in *Q. robur*, in lowland Southwest, may mean that this species becomes the dominant planted species for forestry purposes (Broadmeadow 2004). However, this too may be questionable, as increased attack from pathogens and consequent 'oak decline' in this species (for more information on oak decline see Brasier 1999) may give a non native species such as *Q. ilex* an advantage, both ecologically and as a forestry species in the future. Although it should be noted that both within its Mediterranean range and in coastal

areas of the UK, *Q. ilex* has itself suffered from attack of oak pathogens such as *Phytophthora* spp.[1] Indeed *Q. ilex* shows morphological plasticity within a wide thermic range (Barbero et al. 1992; Gimeno et al. 2009) and is not only able to decrease *SLA* in response to stress but also exhibits a quick stomatal response to water stress and high temperatures whereby it down regulates photosynthesis until temperatures become more favourable (Gratani, Pesoli, Crescente, Aichner and Larcher 2000).

More specifically the results of the experiments (and observations) detailed here, were aimed at evaluating whether *Q. ilex* had greater carbon sequestration potential and photosynthetic efficiency than *Q. robur* and whether this was also true in each rural, urban and upland environment.

Through the course of the experiments it was found that the environmental effects of the sapling sites was very much site specific and although no direct correlations between climate and edaphic variables were made, those sites with greater macro nutrients and greater soil moisture values had the greatest photosynthetic efficiency and growth in both species although soil fertility was more important for *Q. robur* than *Q. ilex* as suggested by the literature (e.g. Litton et al. 2007).

8.0.4 Summary of main results: saplings and mature trees.

Photosynthetic parameters and leaf traits.

In saplings, *Q. robur* had significantly higher A_n than *Q. ilex* during the summer, and again when all months were taken into account ($F_{(1,495)} = 25.4$, p<0.05)(7.9 $\pm$ 3.5 *Q.robur*, N=247; 6.6 $\pm$ 2.1, *Q. ilex*, N=250 ($\mu mol\ m^{-2}\ s^{-1}$)). However, *Q. ilex* A_n was higher at urban *L* in field grown saplings where soils were drier, had the lowest nutrient status (e.g. soil *N* was 1.93 kg^{-1} compared to the next lowest value of 2.79 at DPF) and saplings were more exposed. This was despite photochemical quenching being lower in *Q. ilex* at this site. *PI* was significantly lower in *Q. robur* than *Q. ilex* at rural site B, situated on Dartmoor fringe, having the second lowest air temperatures after the upland site and second driest site after L.

N_{leaf} and *SLA* were generally higher in *Q. robur* than *Q. ilex* in saplings. Highest *SLA* and

[1] In particular, *Phytophtora cinnamomi* (Brasier 1999).

lowest N_{leaf} values were at the two coldest sites (DPF and B). In contrast the urban site L had the lowest *SLA* in both species.

In mature trees, A_n, *SLA* and N_{leaf} were all higher in *Q. robur* although A_n was only significantly higher in *Q. robur* at the urban site in contrast with sapling data. However, when March data was taken into account there were no differences between species (5.2 ± 0.05 *Q. ilex* n=1140; 5.5 ± 0.04 *Q. robur*, n=900 ($\mu mol\ m^{-2}\ s^{-1}$)) although *Q.ilex* A_n was significantly higher at the upland site compared with other sites (6.8 ± 0.05) and was also higher than *Q. robur* at this site, again this is in contrast to sapling data. N_{leaf} was higher in *Q. robur* at the urban site but this difference was not significant, however, C_{leaf} was lower at the urban site (this trend was only significant in *Q. ilex* leaves).

In mature trees, F_v/F_m was higher in *Q. ilex* than *Q. robur* at the rural site, the same at the upland site and lower at the urban site. F_v/F_m was also lower at the urban site compared to the other sites in *Q. ilex*. *PI* however was higher in *Q. ilex* than *Q. robur* at all sites. Within species differences meant that *Q. robur PI* was lower at the upland compared to the other sites. When different dates were compared, F_v/F_m and *PI* was significantly lower at the urban site in the drier June 2010 but increased in June 2011 (accounting for the difference in sample size for each year). For *Q. ilex* mean A_n was low at all sites in June 2010 and remained low at the urban site in June 2011, however March 2011 A_n was significantly higher at all sites for this species. Despite this F_v/F_m was lower during March although F_v/F_m was not affected by drought as June 2010 and June 2011 had similarly high F_v/F_m values. However, *PI* was significantly lower during June 2010 and March 2011 for this species.

Intrinsic Water-use Efficiency ($iWUE$).

Mean intrinsic water use efficiency $iWUE$ was lower in mature trees than in saplings, although maximum $iWUE$ was higher in mature trees. In saplings $iWUE$ was higher at the urban site, L, situated on the top of a south facing slope above Plymouth railway line at Laira in Plymouth. This site had the lowest percentage water content of all sites, (just 19 % in wet soil). L was also low in macronutrients (see chapter 3). The most productive sites were urban D and rural DT both had the highest *N*, *K* and relatively high *P* in soils at these sites. $iWUE$ was greater in pots

than field grown saplings for all sites and species except at L where $iWUE$ was lower in pots, in *Q. ilex* this was accompanied by a down regulation of A_n.

The effects of sun and shade.

For saplings, assimilation rates were higher in sun than shade leaves in both species, while F_v/F_m and *PI* were lower in sun than shade. Specific leaf area (*SLA*), tended to be lower in sun leaves of both species at the urban sites. However, N_{leaf} was greater in sun leaves at urban site D in both species, possibly an affect of atmospheric *N* disposition from nearby traffic at this site. In general there was less distinction between upper and lower foliage in young *Q. robur* saplings.

For mature trees, differences in leaf position in the canopy were more variable than differences between sun and shade, this was mainly due to site specific differences such as their position at the woodland edge (rural), and as parkland trees (urban and upland). Canopy density could also affect the variability within the results i.e. In the urban *Q. ilex* the upper canopy was more open and therefore there was less distinction between sun and shade within middle and upper canopy levels. Despite inconsistencies in sampling design, sun leaves had lower *SLA* and lower N_{leaf} than shade in both species although these differences were more pronounced in *Q. robur*. The same leaf traits were also lower in upper canopy leaves although the distinction was greater in shade leaves at this level of the canopy, possibly due to higher light availability in the upper canopy as a whole.

Differences in Biomass for each species.

In saplings, *Q. ilex* had greater total biomass than *Q. robur* at all sites except the upland site. The largest differences between species were found at rural site B and urban site L.

In mature trees *Q. robur* had the largest biomass at rural and urban sites but contrary to sapling data, *Q. ilex* biomass was greater than *Q. robur* at the upland site. However, the variability in age between sites makes it difficult to make accurate comparisons between sites for individual species.

Q. ilex saplings allocated more biomass to foliage and branches, while *Q. robur* allocated more

to stem and roots.

8.1 Comparisons with the literature.

8.1.1 Ontogenetic Differences.

Sapling A_n was higher than mature trees (4.36 $\pm$ 2.5, 5.47 $\pm$ 3.53 ($\mu mol\ m^{-2}\ s^{-1}$) mature) compared with (7.37 $\pm$ 4.29, 9.15 $\pm$ 4.56 ($\mu mol\ m^{-2}\ s^{-1}$) sapling) maximum A_n was higher in mature trees 9.22 ($\mu mol\ m^{-2}\ s^{-1}$) *Q. ilex*, 21.68 ($\mu mol\ m^{-2}\ s^{-1}$) *Q. robur* compared with 17 *Q. ilex* and 20.5 *Q. robur* in saplings. Maximum photosynthesis in *Q. rubra* was between three and six times higher in mature trees compared to young saplings depending on annual precipitation (as sapling photosynthesis declined during drought) (Cavender-Bares and Bazzaz 2000). Intrinsic water use, $iWUE$ was higher in *Q. ilex* saplings but lower in *Q. ilex* as mature trees. However the urban environment had the effect of lowering $iWUE$ in both life stages in both species. N content and LMA were also higher in mature tree leaves compared with saplings, similar results have previously been found in *Q. ilex* and deciduous oaks (Cavender-Bares and Bazzaz 2000; Mediavilla and Escudero 2003). In contrast to my results, comparisons between evergreen and deciduous oaks growing in the Mediterranean showed that mean A_n was not much different between *Q. ilex* seedlings and mature trees whereas in deciduous oaks, seedling A_n was much lower than in adult trees (Mediavilla and Escudero 2003). Deciduous oaks also had higher leaf N_{mass} in adult trees compared with seedlings, whereas there was no difference in leaf N_{mass} between ontogenetic stages in *Q. ilex*. $iWUE$ was also lower in seedlings than adults in both evergreen and deciduous oaks (Mediavilla and Escudero 2003).

Previous research has also shown how seedling assimilation rates in the deciduous *Q. rubra* were a third of the level of mature trees during a wet photosynthetic season, additionally, water use efficiency (WUE) increased during drought as assimilation rates were lowered. Mature trees had a faster response to drought increasing WUE while maintaining assimilation rates, thus showing that mature trees are better able to withstand drought than seedlings due to deeper rooting depths (Cavender-Bares and Bazzaz 2000).

In southern California during drought, evergreen and deciduous oak seedlings aged 1-4 years

had lower F_v/F_m, lower assimilation rates and lower $iWUE$ than mature trees situated nearby, in addition the evergreen *Q. agrifolia* had the lowest F_v/F_m which suggested the evergreen was under more stress than the deciduous *Q. lobata* (Mahall et al. 2009). However, analyses of my own data suggests that recovery from drought is much better if conditions improve the following year. Contradictions in the literature may be due to differences in sampling dates, but also many comparisons between evergreen and deciduous oaks within the literature are conducted in Mediterranean environments where seedlings are more likely to compete under dense evergreen canopies (Sanchez-Gomez et al. 2006).

Environmental effects on leaf traits.

Oak seedlings are especially vulnerable to drought during the first two years of establishment (e.g. Gomez-Aparicio et al. 2008). In my research, this effect was exacerbated by transplanting stress and soil fertility; therefore *Q. robur* saplings were affected by losses at the drier less fertile sites. Leaf N_{area} and *SLA* increased as rainfall decreased (Wright et al. 2004) while *SLA* can also decrease with decreasing mean annual temperatures (MAT) in evergreen broadleaved trees compared to a more conservative response in deciduous species (Wright et al. 2005). Low N_{leaf} and low *SLA* corresponded with low soil fertility and vice versa and when measured on a mass basis was related to MAT (Ordpnez et al. 2009). Therefore response in leaf traits of the saplings measured within my research show evidence of complex intrinsic and extrinsic responses in both species, the Mediterranean evergreen increases N_{leaf} at urban locations however this is accented when nutrient supply is good despite this species in generally having a more conservative response to nutrient supply (Valledares et al. 2000).

Differences in Biomass between species.

Q. ilex was only stressed at the upland site and reduced allocation to $LWFt$ was evidence of this. Allocation to root was higher in *Q. robur* but interestingly as $LWFt$ increased $RWFt$ decreased suggesting trade-offs in allocation patterns. However, ontogeny can be a factor, when small, seedlings have a greater $LMFt$ in order to improve carbon assimilation and *RGR*. As they grow allocation to $LWFt$ decreases, with faster growing species having greater $LWFt$ (Poorter et al. 2012).

In general tree seedlings and young trees tend to allocate more of their biomass to leaves (Villar et al. 2006). The percentage that tree saplings may allocate to foliage has been estimated at up to 46%, dropping to just 6% in mature trees (Poorter and Evans 1998). Increased allocation to foliage in young trees can also be a reponse to stress factors (e.g. Niinemets 2010). A young tree may be more susceptible to short periods of stress than a mature tree that has more non structural carbon reserves to draw upon during such events (Niinemets 2010).

In theory plants should allocate carbon in order to increase productivity, therefore allocating to the fraction that will enable greater acquisition of a limiting resource (Weiner 2004). It follows that under low nutrient conditions root biomass should increase (Weiner 2004). However ontogenetic differences show that saplings will allocate more to roots and invest more resources into shoots as they mature (Weiner 2004). Shoot growth is more affected by drought than root growth in oaks (Arend et al. 2011). Young oaks adapt to drought by restricting canopy water loss through stomatal closure and as they mature increasing root depth, therefore ontogeny is important and this may change with age and provenance (Arend et al. 2011). Drought sensitivity was greater in more northerly provenance's of the same species in *Q. petrea*, *Q. robur* and *Q. pubescens*) (Arend et al. 2011). *Betula pendula* seedlings have been shown to alter allocation in roots and shoots in response to nutrients (Ericsson et al. 1996). To conclude, a review analysing data from the literature found that although *LMF* increased with nutrients, *SLA* was affected more, decreasing with increasing shade by 170% more than the corresponding change in *LMF* suggesting that plants are better able to change their morphology in response to environmental change than they are their allocation (Poorter et al. 2012).

Photo-inhibition.

Diurnal rhythms using the light unit suggested that down regulation of photosynthesis during the summer was driven by temperature. Similar results were found by Epron et al. (1992) in *Q. petrea* while another study showed *Q. robur* and *Q. petrea* were able to maintain assimilation rates and photochemical efficiency despite stomatal closure during summer drought (Epron and Dreyer 1993*a*). In contrast, *Q. ilex* winter data showed increased winter assimilation rates coupled with reduced photochemical quenching (F_v/F_m). Although increased photosynthesis

during mild winter temperatures is common in *Q. ilex*, leaves can become stressed at temperatures below 15 and above $35°C$ and combinations of high light and low temperatures can induce photo-inhibition (Larcher 2000).

Mature *Q. ilex*, growing naturally in Spain experienced reduced F_v/F_m during winter, a reduction in F_v/F_m can be attributed to a reduction in photochemical quenching in PSII and in this paper was attributed to photo inhibitory processes in the xanthophyll cycle activated without water stress (Martinez-Ferri et al. 2004). Increased evidence of photo inhibitory processes and resulting lowered F_v/F_m has also been shown in *Q. suber* growing in Southern Portugal where frosts are rare and average winter temperatures rarely dip below zero (Garcia-Plazaola et al. 1997). A similar response was also found in *Q. ilex* growing in Spain, (where average winter temperatures where around $3.5°C$) (GarcIa-Plazaola et al. 1999). In agreement with my research, maximum assimilation rates were not down regulated in response to the milder winter temperatures (between 5-$10°C$) despite reduced photochemical efficiency, evidence of a photo protective response (GarcIa-Plazaola et al. 1999). However, in my research, sapling data did show that at the upland site at Princetown, Dartmoor, and at the control site, monthly assimilation was reduced when mean temperature fell below 5 $^{\circ|}C$ (Figures 4.6, 4.9 and 4.9a and Figure 3.3 d for upland climate variables).

Low temperatures reduce assimilation in *Q. ilex* (Larcher 2000). In this research mean A_n was reduced in *Q. ilex* during winter at the upland site and (to a lesser extent) at the Dartmoor fringe site Broadley in saplings. Whereas, in mature trees, mean A_n was higher in March at all sites even though mean temperatures for that month were in fact below the optimum for *Q. ilex* assimilation at all sites ($6.2 - 8.7°C$). This suggests that drought and higher temperatures exert more control over *Q. ilex* than cold stress, or that *Q. ilex* is able to withstand temperatures as low as 5°C before photosynthesis is down regulated. As thylakoid membranes are sensitive to changes in the environment, changes in photochemistry are early indicators of temperature stress (Larcher 2000) and in mature trees F_v/F_m is reduced more so at the upland site, high light in conjunction with low temperatures can cause photo protective mechanisms thus reducing photosynthetic efficiency (Larcher 2000). On cold days, light harvesting is maintained to en-

sure high concentrations of caratenoids involved in the xanthophyll cycle (as a photo-protective measure), this in turn causes a consequent reduction in PSII efficiency. However, drought may also modify the effect of cold on growth (Crescente et al. 2002).

According to Larcher (2000), *Q. ilex* has a cold frost tolerance of $-15°C$ before visual damage occurs, compared to other Mediterranean vegetation. Interestingly in my research the June 2010 data (the drier June) showed no reduction in F_v/F_m at rural and upland sites despite a small reduction at the urban site, whereas *PI* is reduced at all sites during this drier June compared to June 2011 which may be evidence that the incorporation of different stages of the photochemical process in PSII recorded in the performance index *PI* (see chapter 2 section 2.3.6 for full interpretation of *PI*) is better at revealing signs of drought stress in plants.

In fact, despite F_v/F_m, as a measure of photosystem II efficiency being widely cited in the literature to quantify the effects of drought, the less often used, performance index (*PI*) (as a measure of vitality) has been shown as more susceptible to drought stress than maximum photochemical quenching (F_v/F_m) a phenomenon that has been shown in wheat (Zivcak et al. 2008). Further, *PI* is a useful but little used plant stress parameter, but has been useful in quantifying decreased vitality in Beech leaves subjected to ozone pollution (Clark et al. 2000) and has also been used to indicate urban tree health (Hermans et al. 2003) although it was less useful than direct chlorophyll measurements at assessing health of natural oak stands suffering from defoliation (Rossini et al. 2006).

This suggests that use of both F_v/F_m and *PI* in determining the complex interactions between temperature and water stress would be useful addition to tree productivity research.

8.2 Conclusion.

Despite the ability of our native *Q. robur* to reach maximum assimilations rates in response to high summer temperatures, the trade off in water use efficiency leaves it vulnerable to drought. In contrast the evergreen *Q. ilex* is able to increase productivity in the mild oceanic climate of Southwest UK and the trade offs in maximum assimilation rates incurred from summer down regulation of the photosynthetic process mean *Q. ilex* is able to absorb more atmospheric carbon

over time than *Q. robur* at least at the sapling stage when it invests in greater foliage allocation. Greater productivity in *Q. ilex* at Castle Drogo, 300masl, is further evidence of the environmental plasticity of this species although for now at least *Q. robur* is still at an advantage on the high moor where instead it has to compete with *Q. petrea* on less fertile soils.

Finally, in my research the control site polytunnel saplings *Q. ilex* grew more quickly than *Q. robur*, *Q. robur* saplings suffered stress in the from *Erysiphe alphitoides* (powdery mildew) which almost halved max A_n in affected leaves. A reduction in maximum assimilation rates of around 30-40% in infected oak leaves was also found by (Hajji et al. 2009). In fact, *Q. robur* and in the more oceanic/humid areas of *Q. ilex* Mediterranean range, both these species are affected by pathogen attack (as discussed above). In mature trees, *Quercs ilex* did surprisingly well at castle Drogo upland site, where *Q. robur* was suffering from defoliation. The prescence of thin, nutrient poor soils seem to be reducing *Q. robur* resilience to attack at this site.

Further, *Q. ilex* is extending its range further north into central Europe and *Q. ilex* (sub sp. *ilex*) is already found along the Atlantic coast of France as far as Brittany (Barbero et al. 1992). However, caution should be taken when planting non native species, due to the competitive ability of a plant to colonise areas and out compete native vegetation for space, authors such as Peterken (2001) suggest *Q. ilex* is already a potential invasive species and the UK organisation,'Plantlife' would like to see this species added to Schedule 9 of the Wildlife and Countryside Act in England and Wales (Plantlife 2010).

8.3 Limitations of this research

Q. ilex always had a nutritional advantage due to being pot grown compared to *Q. robur* bare root stock. However, in terms of cold hardening, *Q. robur* had the advantage. In turn, potted *Q. ilex* had greater productivity at sites where soil macro nutrients were scarce which would suggest *Q. ilex* can tolerate less fertile soils.

Planting one year saplings without watering in for the first season caused survival rates to be low at the more stressful sites for both species. With hindsight it may have been better to water in for the first Spring before leaving to take measurements the following growing season.

In the mature tree experiment, although each site interspecies differences in age were minimum, the variability in age between sites and multi stem *Q. ilex* compared to single stemmed mature *Q. robur* at the upland site made biomass estimates more difficult for this life stage. Additionally, lack of mature *Q. ilex* at Princetown, Dartmoor meant the mature tree upland site was 100m lower in altitude than the sapling upland site, therefore comparisons between ontogenetic stage were also difficult.

8.4 Further Work.

In order to fully evaluate productivity and invasiveness potential of *Querus ilex*, germination success in relation to environment studies would need to be completed alongside more intensive work to draw out the effects of pollution from the effects of water stress. Closer analyses of pollution concentrations would make the distinction between the rural and urban environment easier to analyse. Although monthly climatic variables and soil information was gathered from sapling data. The number of different variables made it difficult to find meaningful relationships using multivariate analysis. It would be interesting to conduct a similar experiment using open top chambers in conjunction with field analyses. Additionally, there is evidence from this thesis to suggest that despite *Q. robur* having a wider European distribution, *Q. ilex* drought tolerance combined with its ability to withstand colder temperatures, warrants more research on this species growing within the UK as the majority of literature on this subject is mainly conducted within this species Mediterranean range.

Further work on biodiversity related projects is also advisable, analyses of biodiversity in the English versus Holm oak currently found in the literature found just five phytaphagus insect on *Q. ilex* compared to 243 on *Q. robur* (Kennedy and Southward 1984). The same authors reanalysed 20 years later and although the number of insects found on *Q. robur* had increased by 40, the number found on *Q. ilex* had increased to 89 (Southward et al. 2004) a nearly 20 fold increase.

Appendix A

Soils Schedules

[all schedules are taken direct from Geography lab schedules (Kev Sloman, 2009)].

A.1 TOTAL N

A.1.1 Total Nitrogen determination of soils.

The soil is digested under reflux with concentrated sulphuric acid in the presence of sodium sulphate (to raise the temperature of digestion) and copper catalyst (to promote oxidation of organic matter) to convert nitrogen compounds present to ammonium sulphate. The ammonia of the digest solution is then determined by continuous air-segmented flow colorimetry in which the sample is reacted with salicylate and dichloro-isocyanuric acid to produce a blue complex measured at 660nm. Nitroprusside is used as a catalyst.

Reagents Sulphuric acid (d20 1.84) Catalyst mixture. Mix together 200g of sodium sulphate, anhydrous and 6.0g of copper sulphate, pentahydrate.

Kjeldahl copper catalyst tablets. Each tablet contains 1g sodium sulphate and the equivalent of 0.1g copper II sulphate.

Method Allow to soak overnight a series of digestion tubes containing 10% sulphuric acid. There should be one tube per sample and two for blanks per batch.

Rinse the tubes out with distilled water and allow to dry.
Carefully weigh about 0.50g of air-dried, $< 1.0mm$ soil into a dry digestion tube. Record the exact weight of the soil to the nearest 0.0001g.
Add $1.0 \pm 0.05g$ of catalyst mixture (or one Kjeldahl copper catalyst tablet).

Cautiously add $10.0 \pm 0.5ml$ of sulphuric acid (d20 1.84).

Swirl gently to mix and insert the tube into the digestion block.

Repeat this procedure for all the samples and add two blanks (ie tubes with reagents only).

Programme the digestion block to gently heat to $200°C$ to remove any water present.

Increase the temperature to allow the contents of the tubes to boil under reflux. Maintain this temperature for 4 hours.

When digestion has completed allow the tubes to cool before removing them.

Carefully add $50ml$ distilled water to the tube. Ensure the tube is pointing away from the operator and anyone else nearby as the addition of water may cause the contents of the tube to react violently.

Transfer the contents to a $100ml$ volumetric flask and dilute to volume with water.

This diluted sample should be filtered or centrifuged prior to storage. Use a Whatman No.542 filter paper. Store in a polyethylene bottle in a fridge if not analysing immediately.

Analyse the ammonia on the Bran and Luebbe Autoanalyser.

Dilute as described in note 1 below.

When preparing the standard solutions, ensure they are acidified to the same concentration as the samples. The base matrix should contain $10.0ml$ H_2SO_4 per $100ml$. This must be diluted to the same extent as the samples prior to making up the standards.

Note 1: Sodium salicylate precipitates in an acid medium. Do not try to neutralise the extracts with sodium hydroxide as this interferes with ammonia chemistry. To overcome the problems with acidified samples the autoanalyser should be run on the low sample range $(0-5mgl^{-1})$ for salicylate chemistry and samples should be diluted 1:20. ie. For a standard 3ml sample, add $0.15ml$ sample to $2.85ml$ water.

Example digest programme (should be varied according to the nature of the sediment)

30 minutes @ $100°C$

30 minutes @ $150°C$

1 hour @ $200°C$

2 hour @ $250^{\circ}C$

1 hour @ $360^{\circ}C$

1 hour @ $375^{\circ}C$

Cooling time = 2 hours

Calculation The initial dry soil weight = *agrams*

Volume of digest = $100ml$

Concentration of Ammonia-N from AA3 = $bmgl-1$

therefore in 100ml extract there is $100b??\mu gNH4+?N$ (this was present in *agrams* soil)

$1Kg$ soil contains $mgtotal-N$ (divide all this by 1000 to obtain grams TN)

For $1.0g$ soil and $100ml$ solution this reduces to;

$1Kg$ soil contains $bx0.10gtotal-N$

A.2 MINERAL N

A.2.1 Soluble Inorganic Nitrate (Mineral N)

Soils should be sampled moist from the field and extraction started within a few hours. Gently crumble the soil and omit any stones. Alternatively store at low temperature (2o - 4oC) without drying or freezing (eg. stored in a sealed plastic bag in a refrigerator). This will restrict mineralization.

Potassium chloride is used to extract soluble and exchangeable nitrate and ammonium ions. Once in the potassium chloride solution, extracts can be stored for up to 2 months in a refrigerator.

Reagent Potassium chloride, $1M$. Dissolve $74.5g$ of KCl in deionised water and make up to 1 litre.

Method Carefully weigh about 2.5g of the moist soil into a 125ml plastic bottle.

Record the exact weight of the soil.

Add 25ml of 1$MKCl$.

Cap the bottle securely and place on a rotary mixer for 1$hour$.

Filter the suspension through a Whatman 541 or Whatman no.1 filter paper. Do not use a membrane filter. If the soil contains a high percentage of clays it may save time if the suspension is first centrifuged for 5$minutes$ at 3500rpm.

Store the filtrate for nitrate analysis and discard the sediment fraction.

Analyse the filtrate using the Bran and Luebbe Autoanalyser 3. Ensure the correct programme is run for the soil nitrate extract.

All standards, wash solutions and dilution waters must be made of the same extractant solution - ie. 1M KCl.

Determine the moisture content of the original soil using the standard method and use the corrected dry soil weight for the calculations.

Calculation The initial dry soil weight = $a?grams$ Volume of KCl extractant = 25ml Concentration of Nitrate from AA3 = $bmgl-1$

therefore in 25ml extract there is $25b??gN$

This was present in $a?grams$ soil 1Kg soil mg N (divide all this by 1000 to obtain grams N)

For 2.5g soil and 25ml KCl this reduces to; 1Kg soil contains b x 0.01g N (Kev Sloman, 2003)

A.3 AVAILABLE PHOSPHATES

A.3.1 Available Phosphate-P extractions from soils

(Brays No.1 extractant)

This method is ideally suited to acidic soils. The selectivity of the extractant is designed to remove the easily acid-soluble P and a portion of the phosphates of Al and Fe. For calcareous soils follow the method using sodium bicarbonate as extractant. The method uses Brays No.1

extractant to remove the available phosphates. This method is sensitive to the soil/extractant ratio, shaking rate and time. It is therefore important that these are kept the same for all samples.

Reagents Bray No.1 extractant. Dissolve $2.22g$ ammonium fluoride, NH_4F, in about $1litre$ of deionised water and add $50ml$ of $1.0NHCl$. Dilute to $2litres$. The solution pH should be 2.65 ± 0.5. Store in a polyethylene bottle.
This solution comprises $0.025NHCl$ and $0.03NNH_4F$.

Reagent A. Dissolve $60g$ ammonium molybdate tetrahydrate in about 200ml distilled water. Add $1.455g$ antimony potassium tartrate and mix to dissolve. Slowly add $700ml$ concentrated sulphuric acid.
Carefully dilute to $1litre$ with distilled water when cooled. This reagent should be dark blue in colour and is stored in an amber glass bottle in a fridge.

Ascorbic acid reagent. Dissolve $33g$ L(+) ascorbic acid in $250ml$ distilled water.

Working Reagent B Pipette $25ml$ of Reagent A into a $1litre$ volumetric flask containing about 800ml deionised water and add 10ml ascorbic acid reagent. Dilute to $1litre$ with deionised water. This solution must be prepared daily.

Method Carefully weigh about $1.0g$ of air-dried, $< 2mm$ soil into a 50ml plastic bottle. Record the exact weight of the soil. Add 10ml of Bray extractant reagent. Cap the bottle securely and place on a rotary mixer for 15 minutes. Leave to stand for 15 minutes. Filter the suspension through a Whatman $No.540$ or 541 filter paper. Alternatively centrifuge the mixture at $3500rpm$ for $5minutes$ and decant off the supernatant fraction for analysis. Measure a $2ml$ aliquot of the filtered fraction into a glass test tube and add 8ml of the working reagent B. Leave to stand for $20minutes$ for the colour to develop. Analyse the prepared sample on a spectrophotometer at $880nm$ wavelength.

Calibration Prepare a set of standard solutions in the range $0 - 8mgl - 1P$ using Bray's reagent as the base matrix. Measure a $2ml$ aliquot of the known standard into a glass test tube

and add 8ml of the working reagent B. Leave to stand for 20$minutes$ for the colour to develop. Analyse the prepared standard on a spectrophotometer at 880nm wavelength. A blank solution can be prepared by pipetting 2ml of the extractant into a test tube and adding 8ml of the working reagent B. Plot a linear calibration curve of concentration of phosphate against absorbance.

Calculation The initial dry soil weight = ??grams Volume of extractant = 10ml Concentration of Phosphate from spectrophotometer = ?$mgl-1$ therefore in 10ml extract there is 10???gP This is present in ??$grams$ soil 1Kg soil contains ?mgP (divide all this by 1000 to obtain grams P)

For 1.0g soil and 10ml extractant this reduces to; 1Kg soil contains ?x0.01gP

A.4 POTASSIUM

A.4.1 The measurement of exchangeable potassium in soils

Reagents Use one of the following acetate preparations;

Ammonium acetate solution, 1M. Add 115ml glacial acetic acid to about 500ml deionised water. In another vessel add 110ml ammonia solution (35%NH_3) to 500ml deionised water. Carefully mix the solutions together in a 2$litre$ volumetric flask and adjust to pH7.0 with acetic acid or ammonia. Dilute to 2$litres$ with deionised water.

Ammonium acetate solution, 1M. Dissolve 77.08g ammonium acetate in 800ml deionised water and dilute to 1$litre$.

Stock standard solution, 1000$mgl-1$ potassium. Dissolve 1.907g potassium chloride in approximately 500ml1M ammonium acetate and dilute to 1$litre$ with 1M ammonium acetate.

Working standard solution, $(10mgl-1K+)$. Dilute the stock standard 1 : 100 with 1M ammonium acetate.

Preparation of extracts Weigh 2.5$g(\pm 0.01g)$ of <2mm air-dried soil into a 125ml HDPE plastic bottle and record the weight.

Add 100ml ammonium acetate and leave for 4 hours or overnight.

Place on a rotary mixer for 30 minutes.

Filter through a Whatman $No.540$ or 542 filter paper. Use vacuum filtration if necessary.

Pour the filtrate into a 100ml volumetric flask and top up to the mark with 1M ammonium acetate. Store in the cleaned plastic bottle.

This extract is used for the determination of exchangeable potassium.

Potassium can be analysed using standard flame photometry or ICP instrumentation.

Notes Store prepared samples in a fridge at $4°C$.

Never leave samples unattended for more than 2 days at room temperature as the extractant can act as a nutrient base for mould growth.

Use the standard extractant as a blank.

If samples require diluting, ensure the matrix in the final sample corresponds with the original extractant.

(Kev Sloman, version 1.3)

Appendix B

Residual Plot Information

B.1 Biomass data: Residual Information

B.1.1 Biomass data; Information on residuals to show assumptions for Linear model affectiveness met

The following figures are residual plots from the biomass fractions: Analysis of residuals can be used to justify the assumptions that need to be met to ensure a linear model is adequate analyisis.

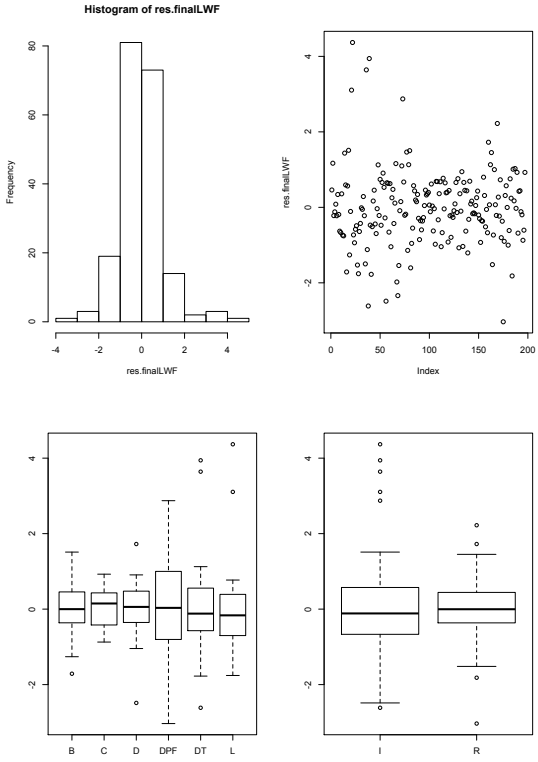


Figure B.1: Residual plot for leaf weight fraction minus control site.

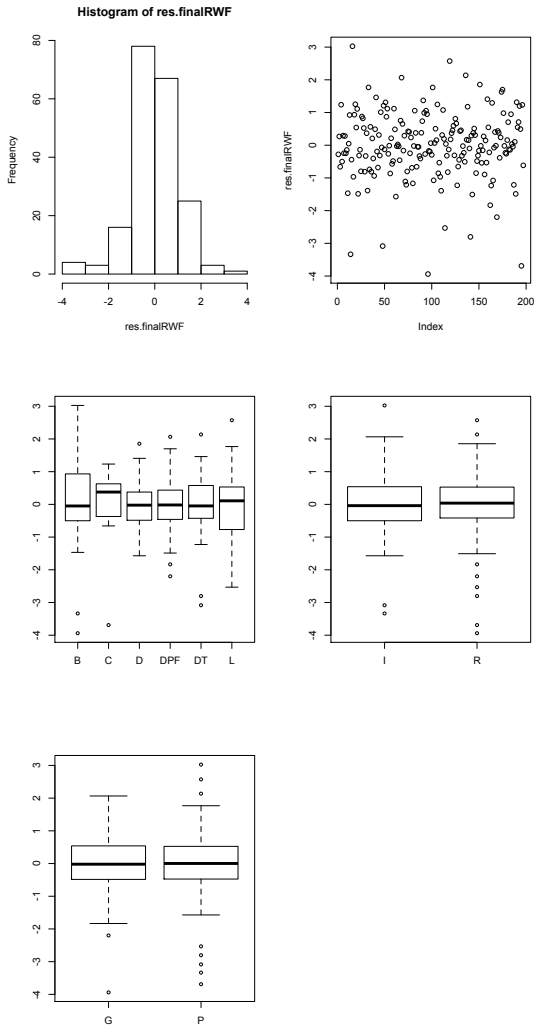


Figure B.2: Residual plot for root weight fraction.

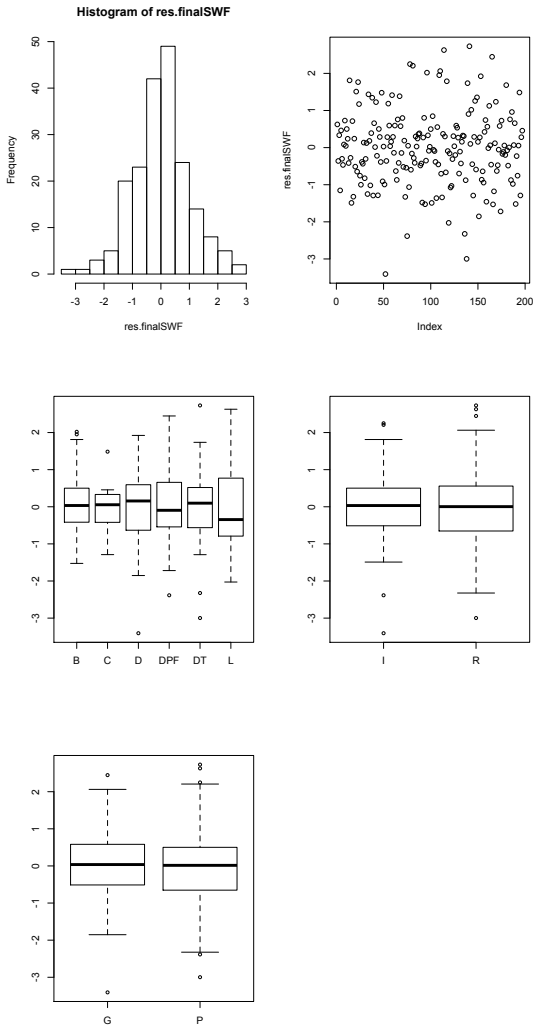


Figure B.3: Residual plot for stem weight fraction.

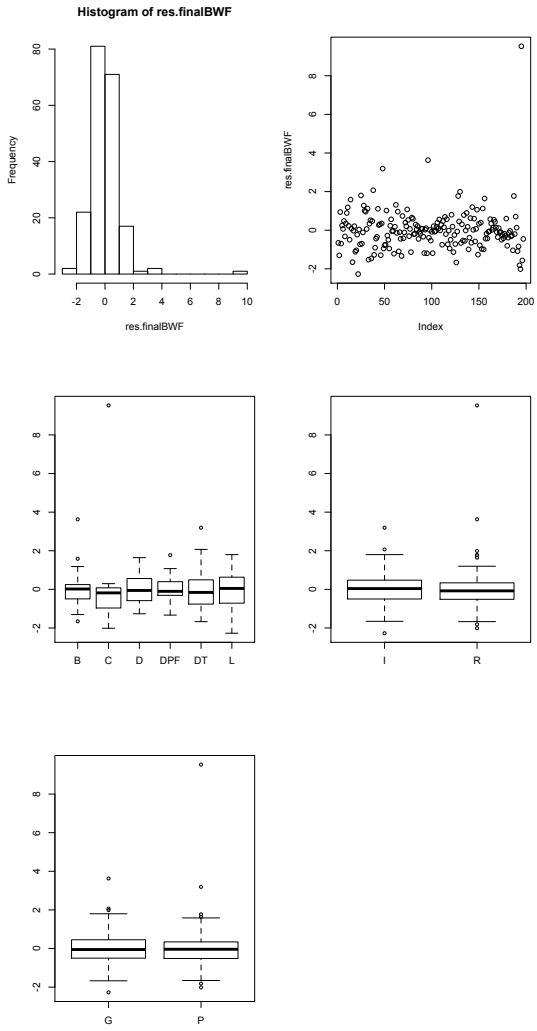


Figure B.4: Residual plot for branch weight fraction.

Appendix C

Interaction Plots.

C.1 Final Above Ground and Total Biomass and fractions Interaction Plots

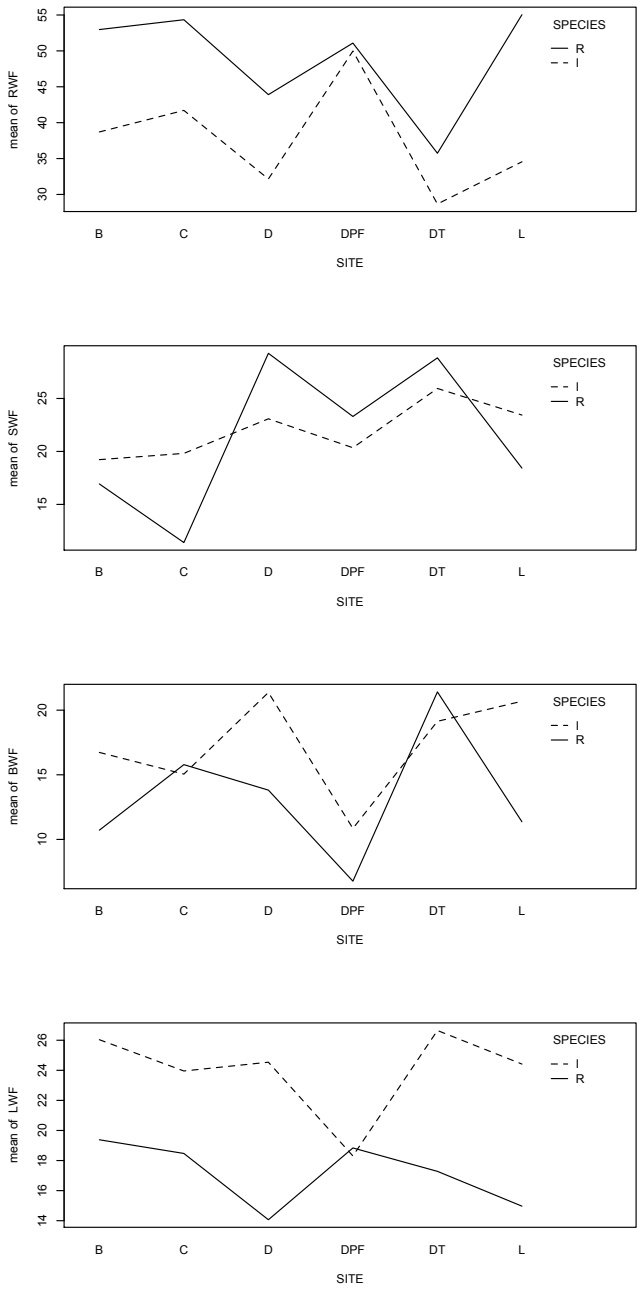


Figure C.1: Interaction plots for biomass fractions; differences between pots and planted saplings.

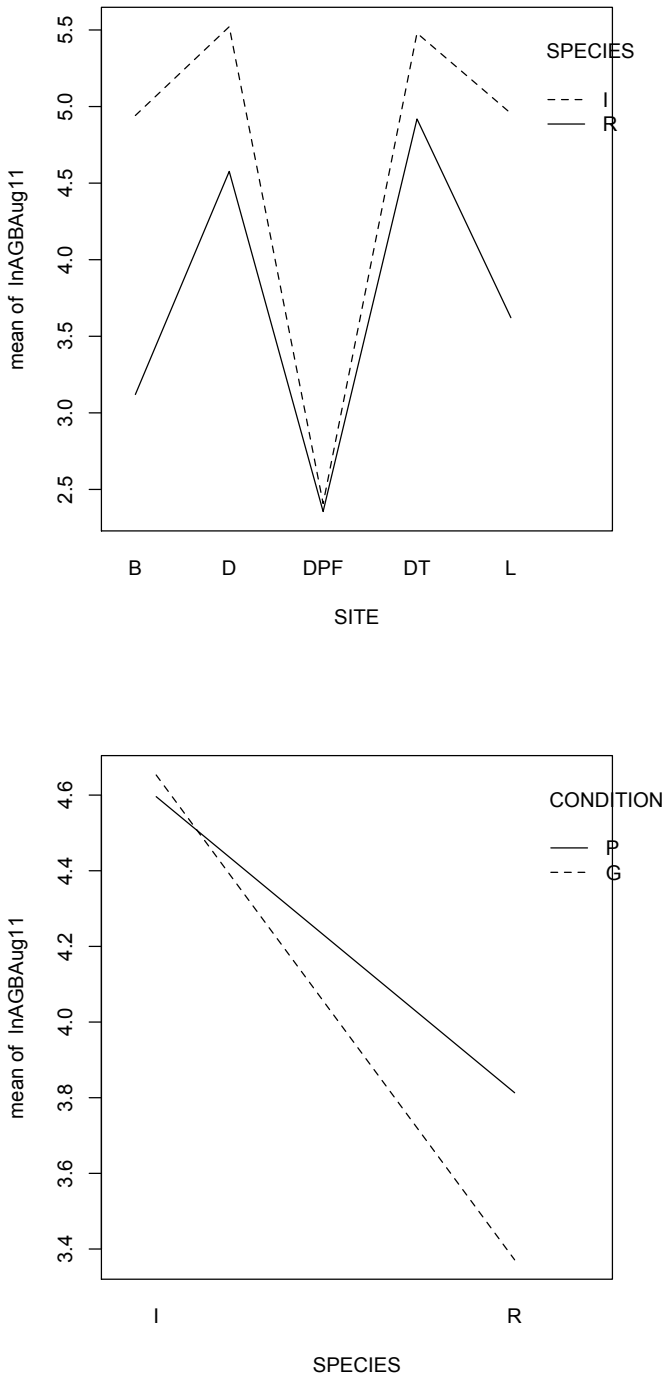


Figure C.2: Above ground biomass, interactions between species and condition.

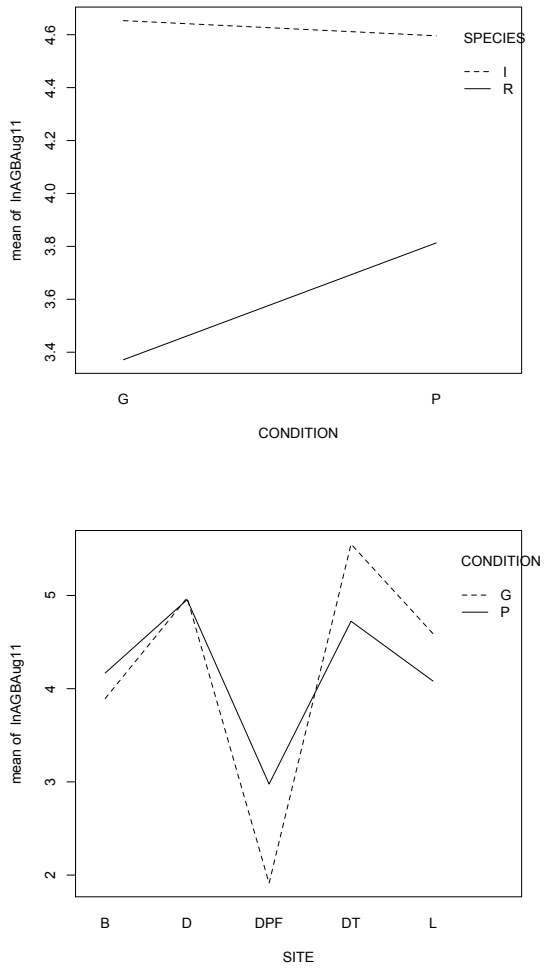


Figure C.3: Above ground biomass interactions with site and condition.

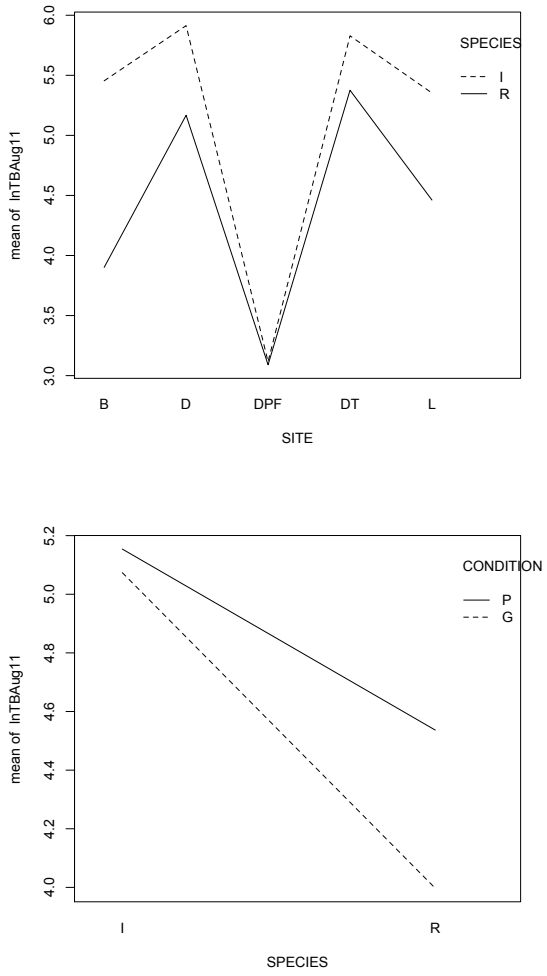


Figure C.4: Total Biomass interactions with species and condition

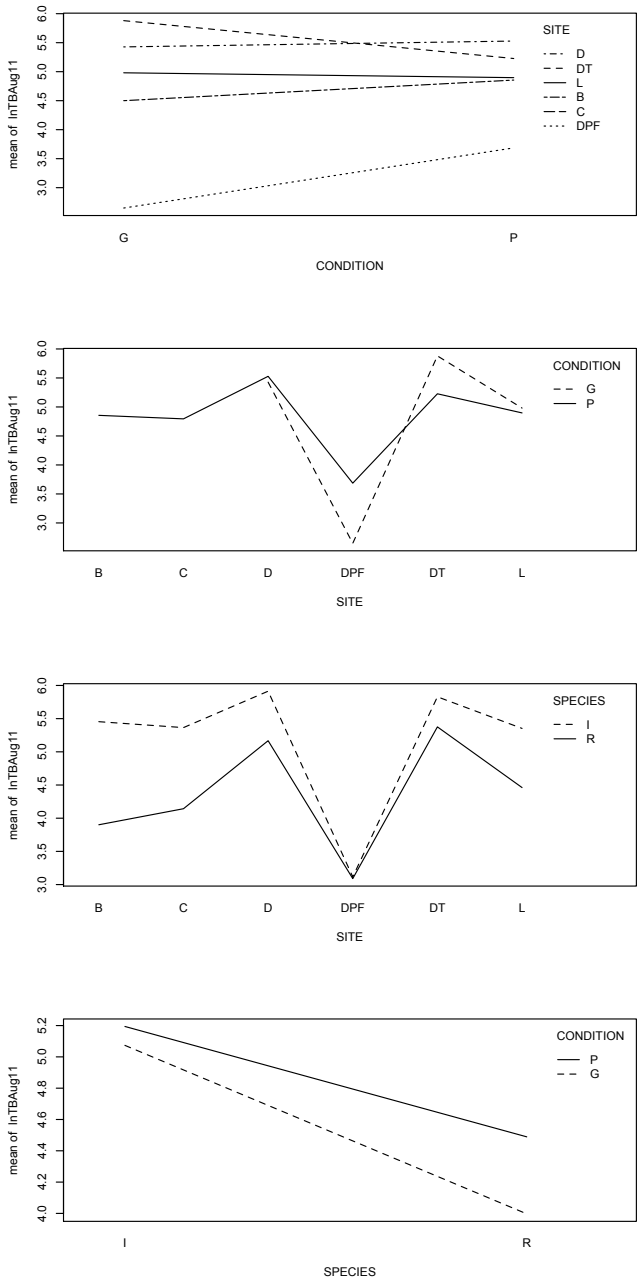


Figure C.5: Total biomass, Interaction plot

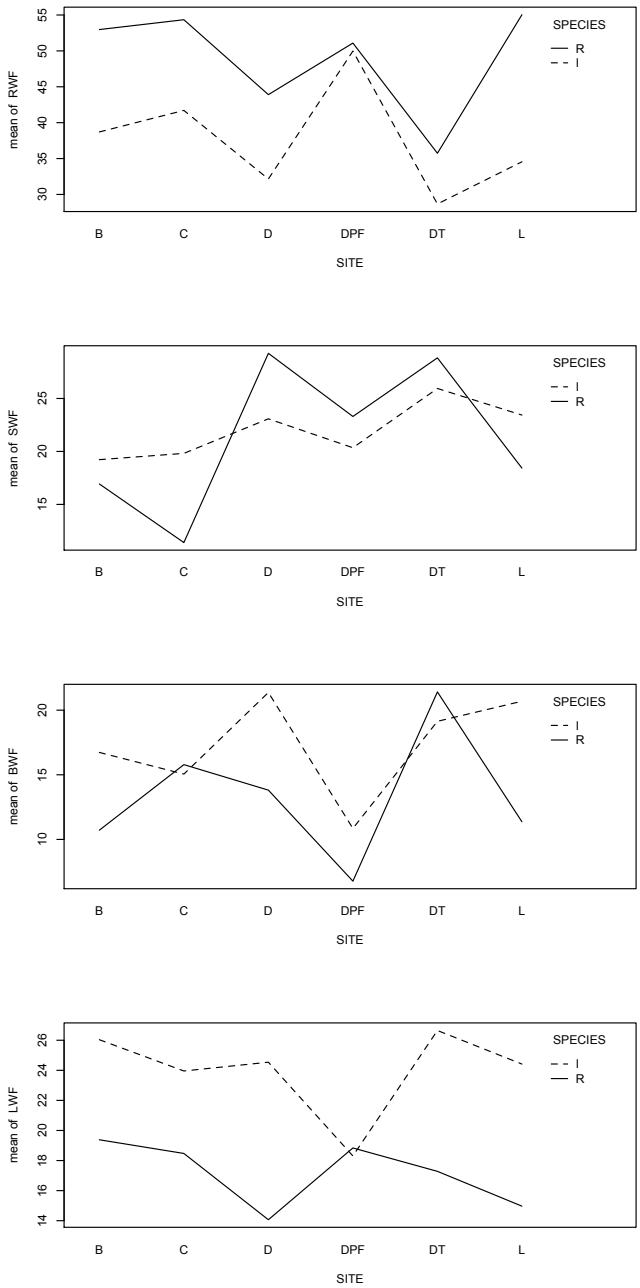


Figure C.6: SPECIES and SITE Interactions for Biomass Fractions LWF, BWF, SWF, RWF, Analysed as percentage of TB.

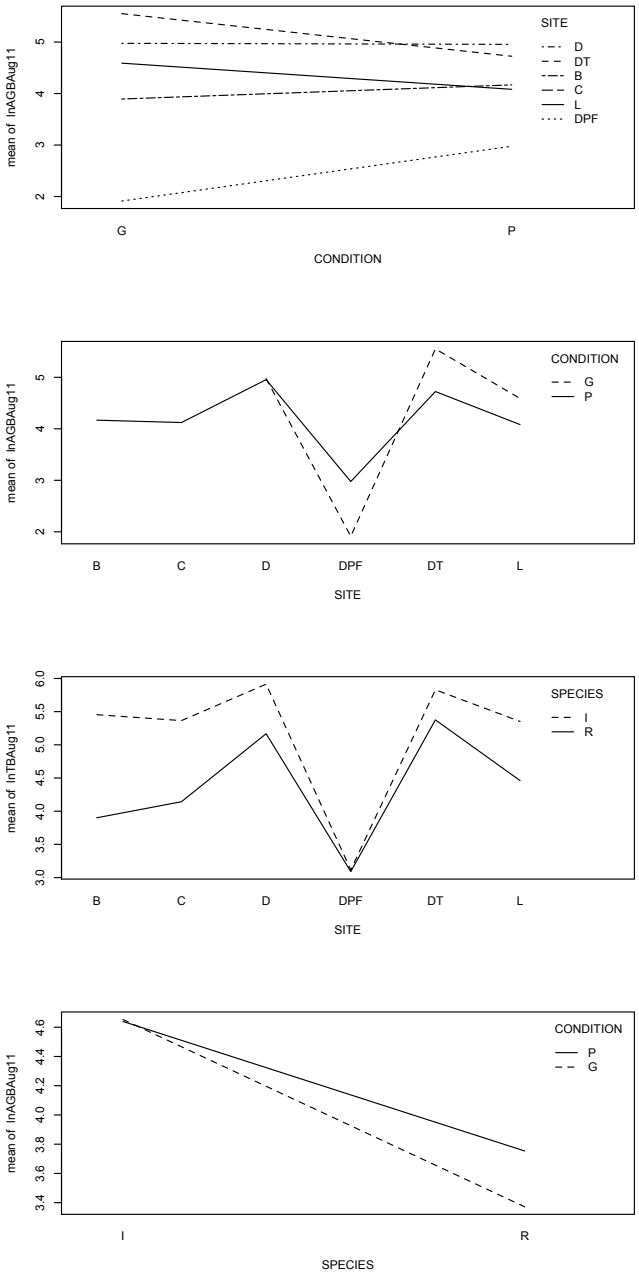


Figure C.7: AGB, interactions between SPECIES and SITE

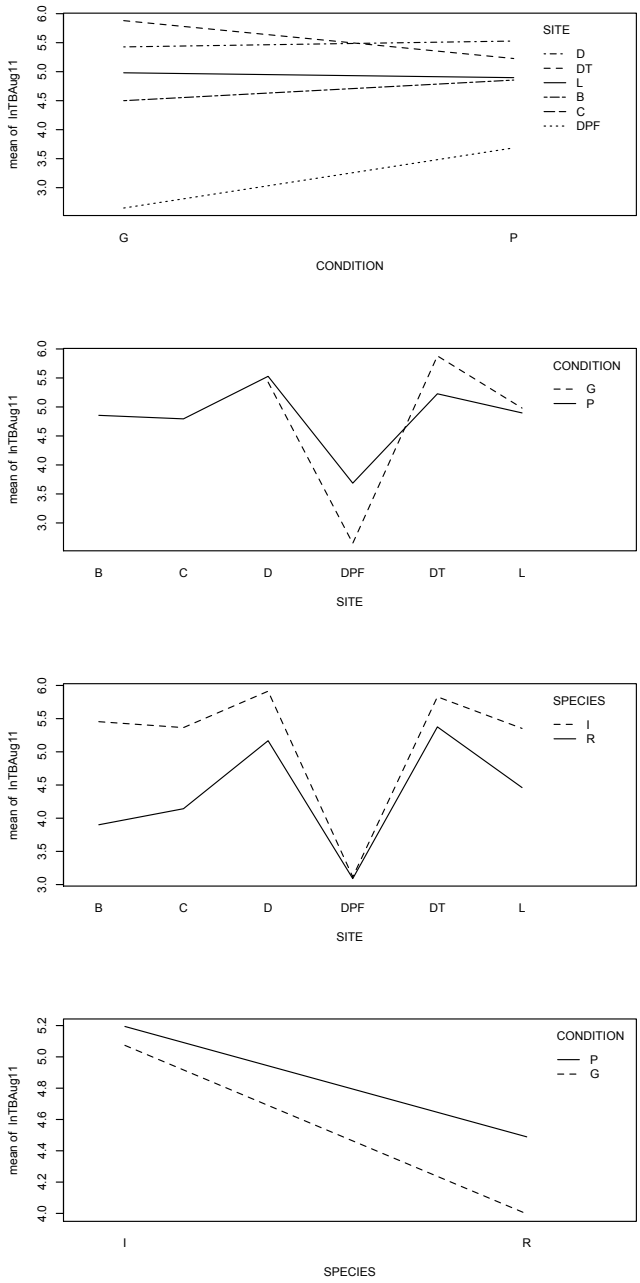


Figure C.8: TB interactions with SPECIES, SITE and CONDITION

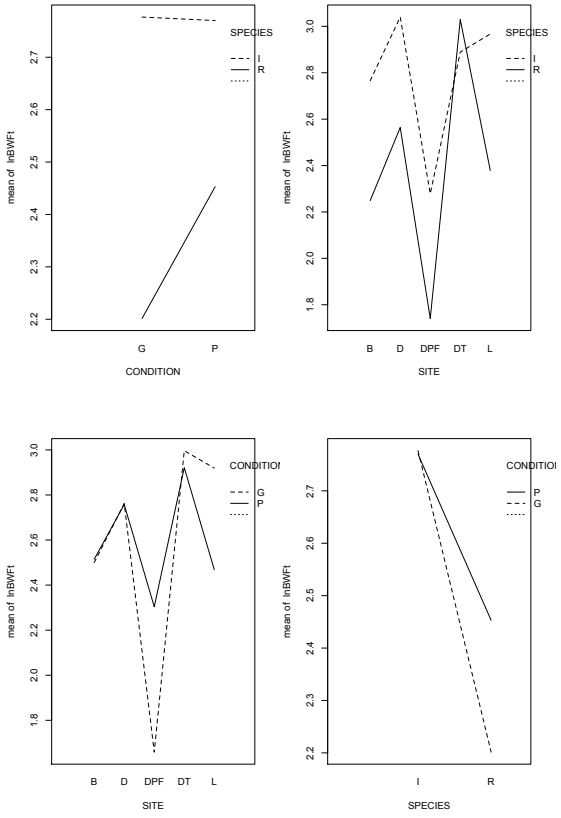


Figure C.9: Percentage allocation of BWFt on logged scale, full interaction plot

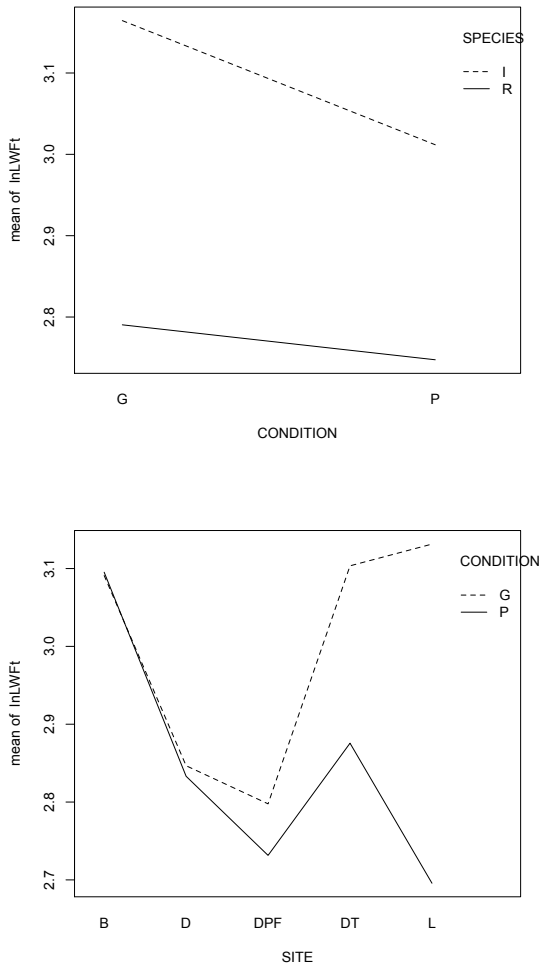


Figure C.10: Percentage allocation of LWFt on logged scale, SPECIES and SITE interactions

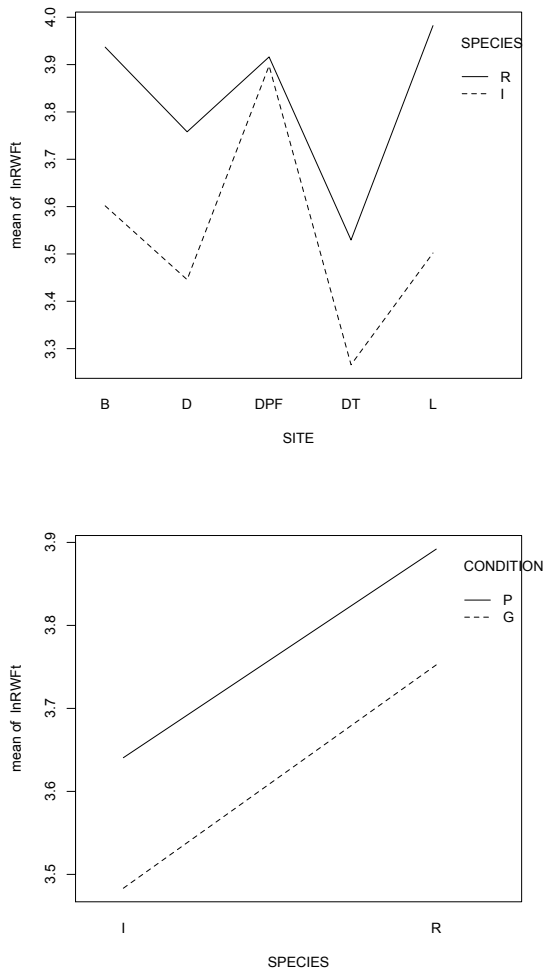


Figure C.11: Percentage allocation of RWFt on logged scale, SPECIES and SITE interactions.

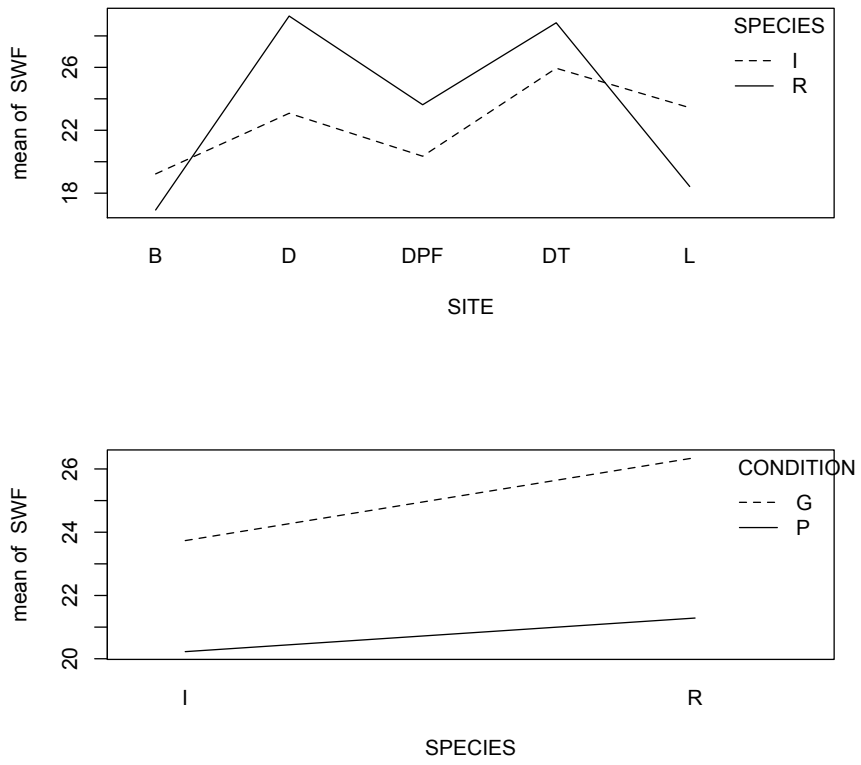
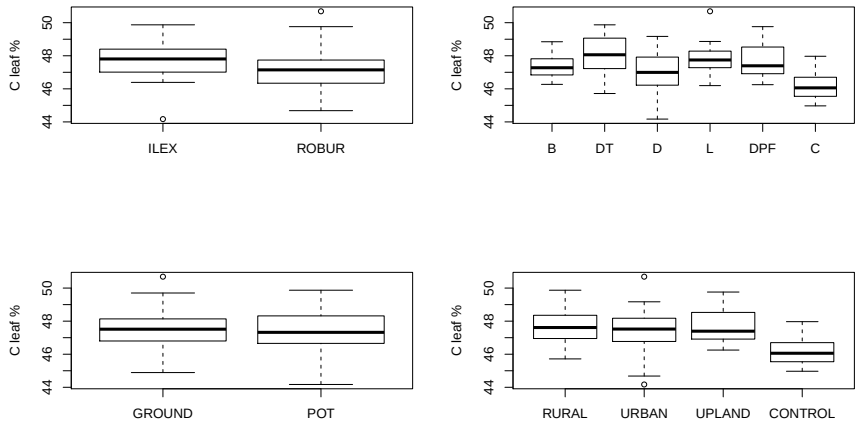


Figure C.12: Percentage allocation of SWFt on logged scale, full interaction plot.

Appendix D

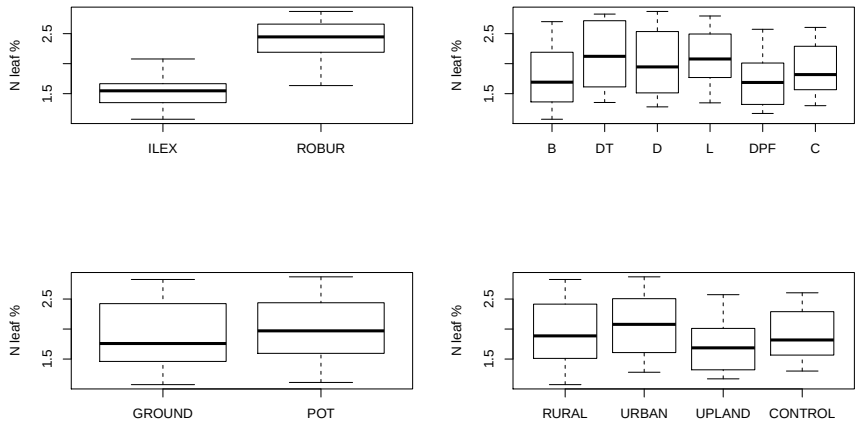
Leaf Trait Interactions with Factors

D.1 Box and Whiskerplots for Leaf traits against factors



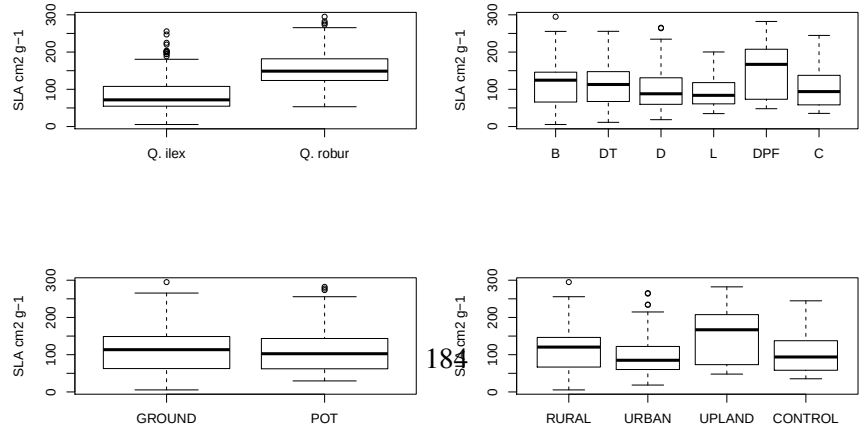
(a) leaf C

Figure D.1: Leaf carbon data for species, site and environment (SPECIES, SITE and CHARACTER) (means ± CI)



(a) leaf N

Figure D.2



(a) SLA

D.1.1 Leaf trait interaction plots

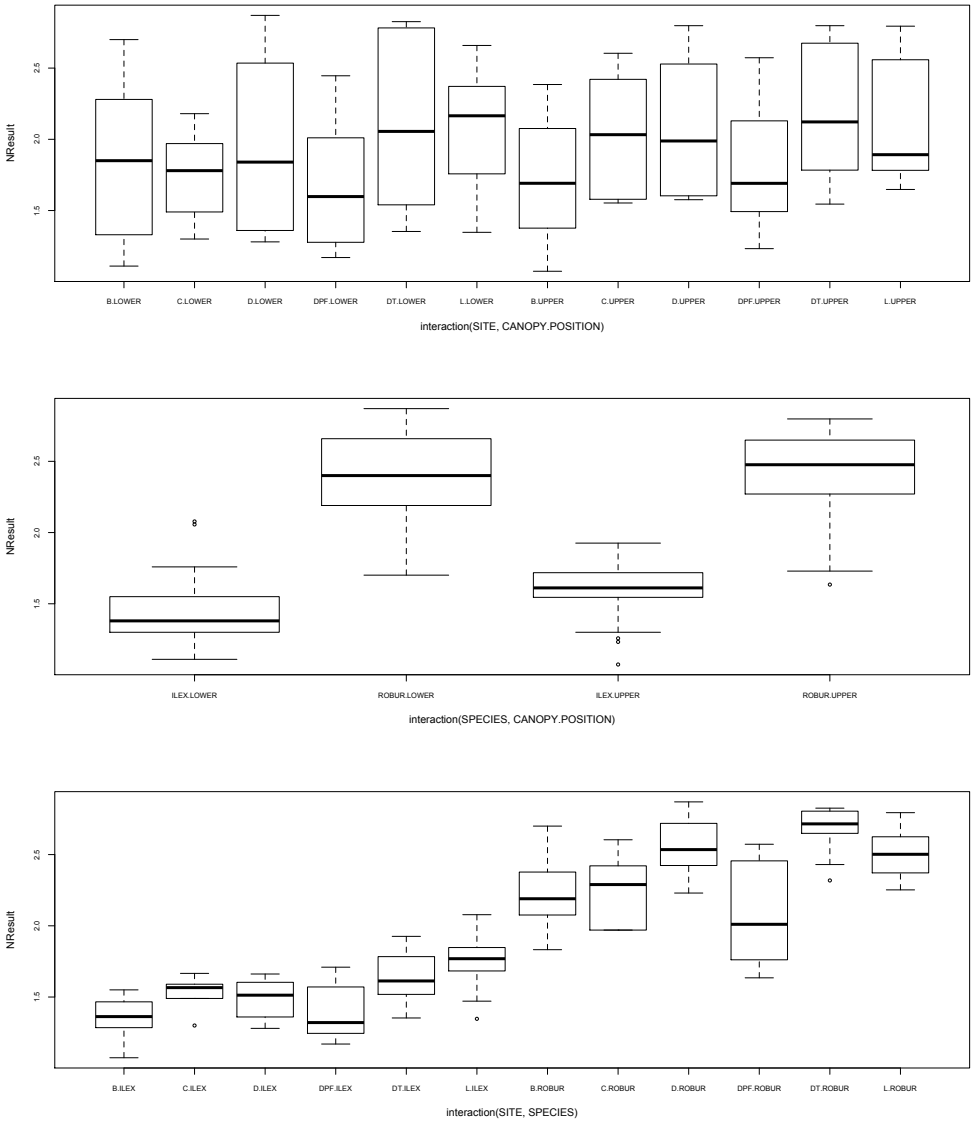


Figure D.4: Leaf N (Nresult in % concentration). Showing Species, site and LP (UPPER/LOWER) interactions (see glossary for site coding) (means $\pm 95\%CI$)

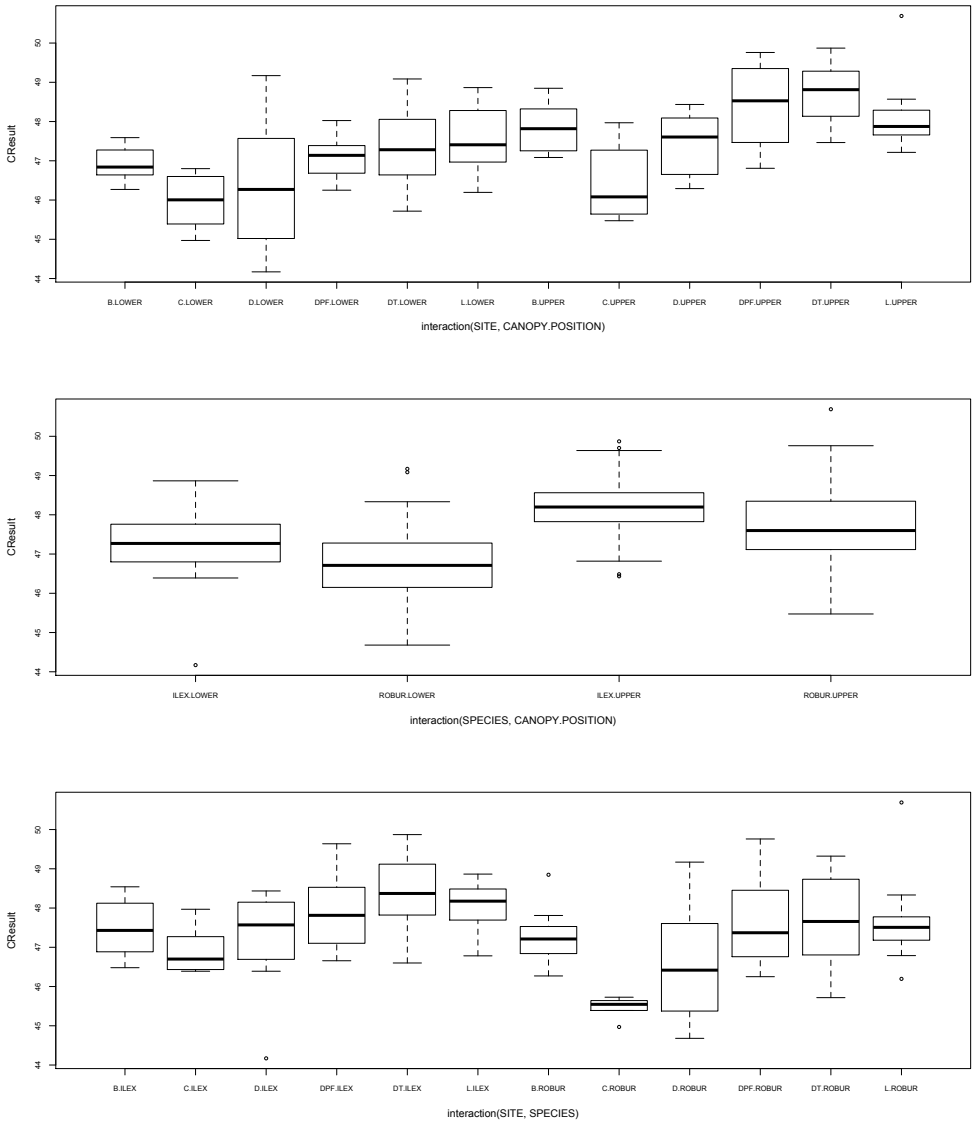


Figure D.5: Leaf C(CResult in % concentration) Showing Species, site and LP (UPPER /LOWER) interaction(means $\pm 95\% CI$)

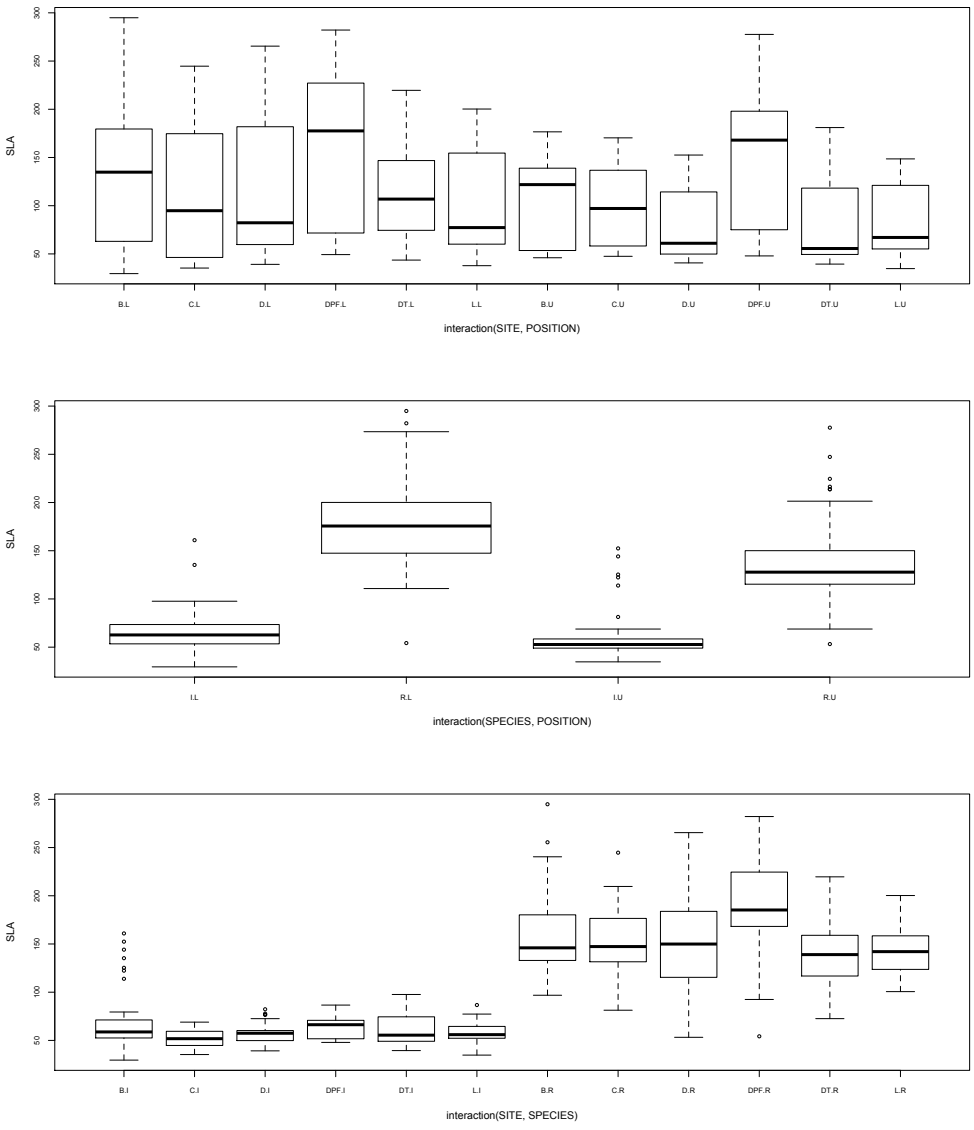


Figure D.6: SLA$(cm^2 g^{-1})$.Showing Species, site and LP (UPPER /LOWER) interaction(means $\pm 95\% CI$)

Appendix E

Biomass: Descriptive statistics and extra graphs

E.1 Actual growth rates.

The following graphs are larger individual growth/time plots for each site without homogeneous y axes, therefore enabling a clearer interpretation of the results shown in chapter 6 Figure 6.1.

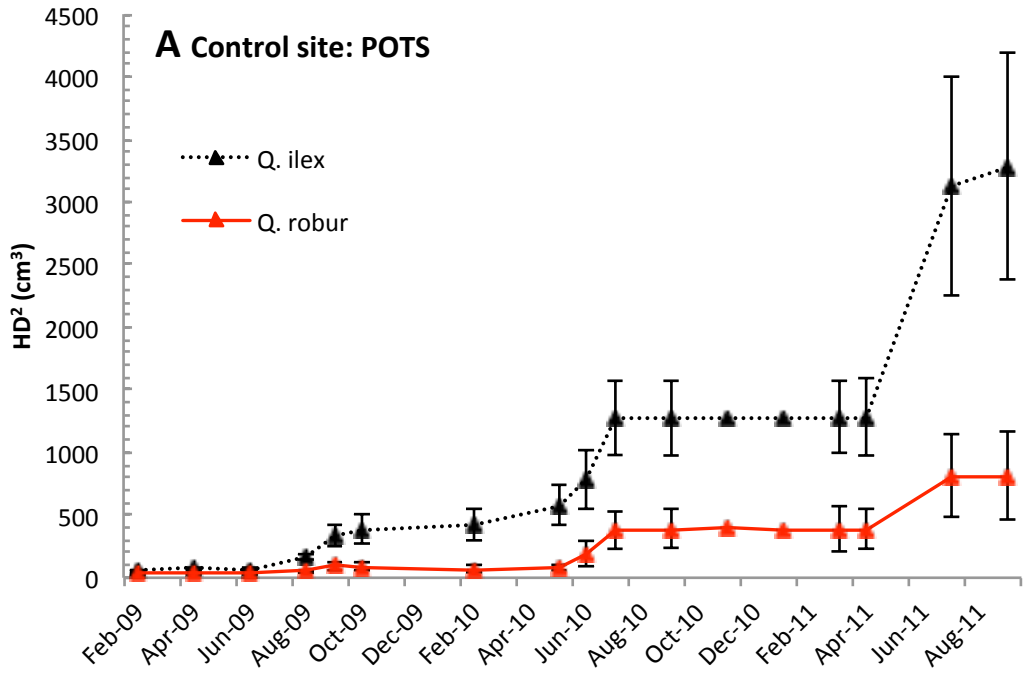


Figure E.1: Monthly growth for control site saplings over the 33 month growth period (means ± 95 % CI).

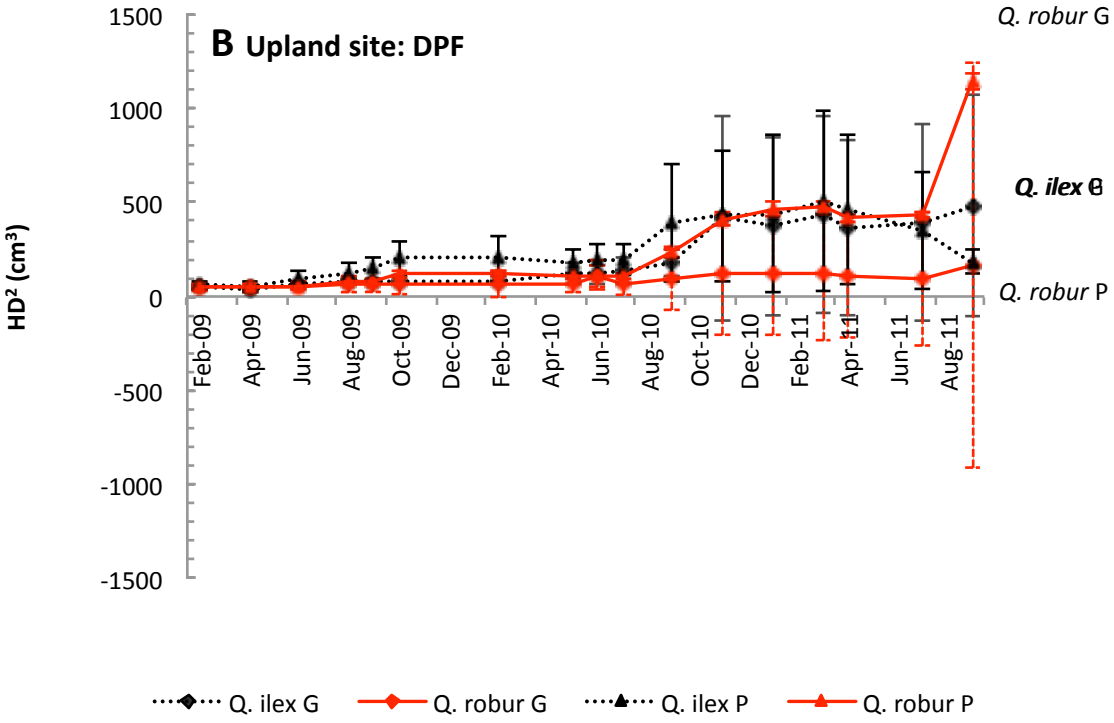


Figure E.2: Monthly growth for upland site saplings over the 33 month growth period (means ± 95 % CI).

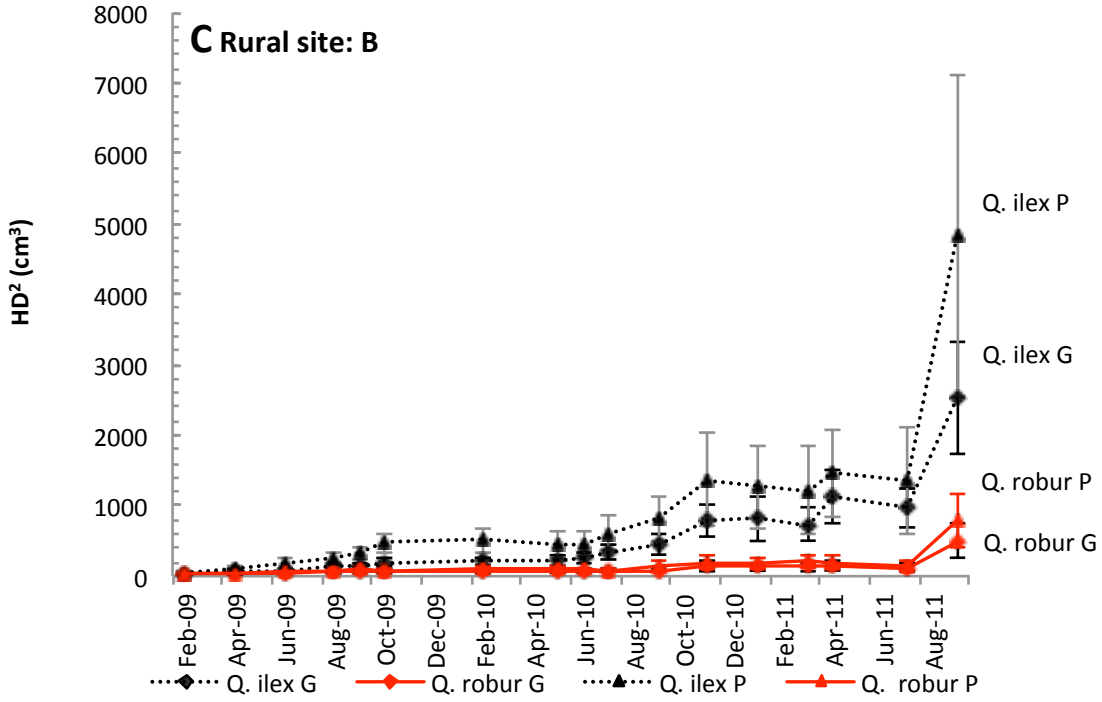


Figure E.3: Monthly growth for rural site B saplings over the 33 month growth period (means ± 95 % CI).

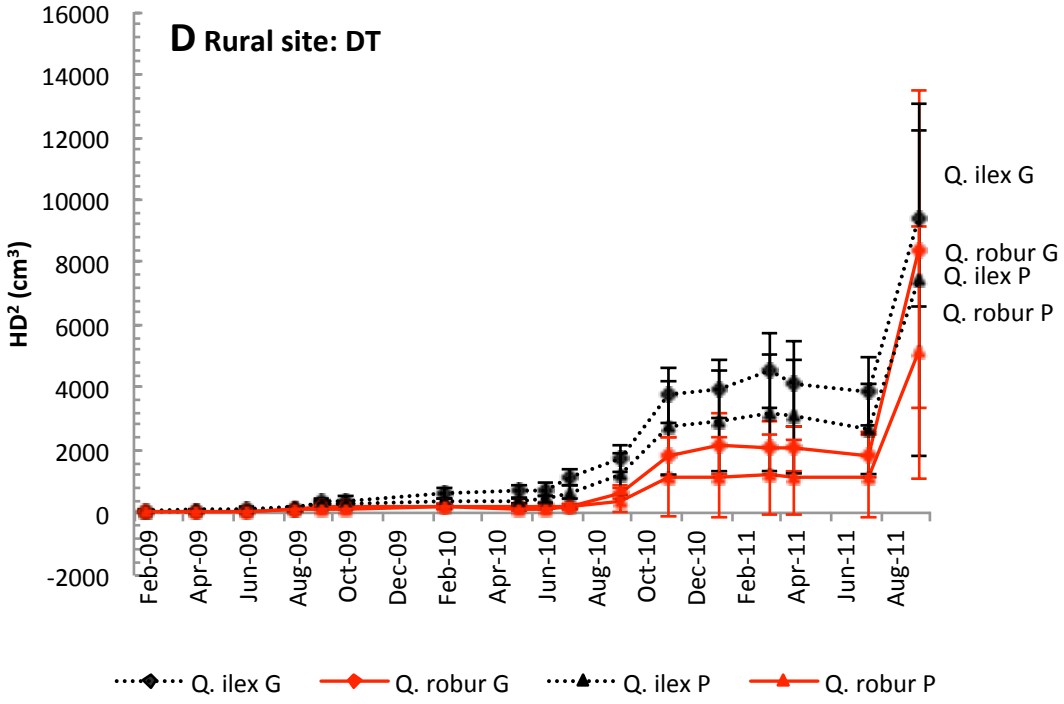


Figure E.4: Monthly growth for rural site DT saplings over the 33 month growth period (means ± 95 % CI).

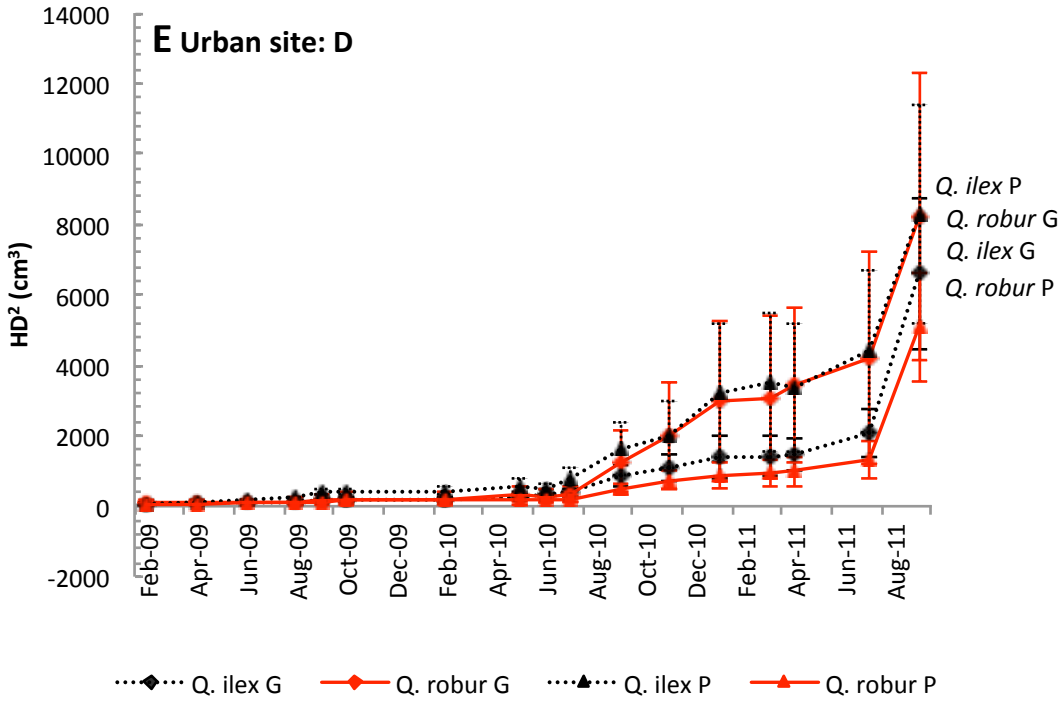


Figure E.5: Monthly growth for urban site D saplings over the 33 month growth period (means ± 95 % CI).

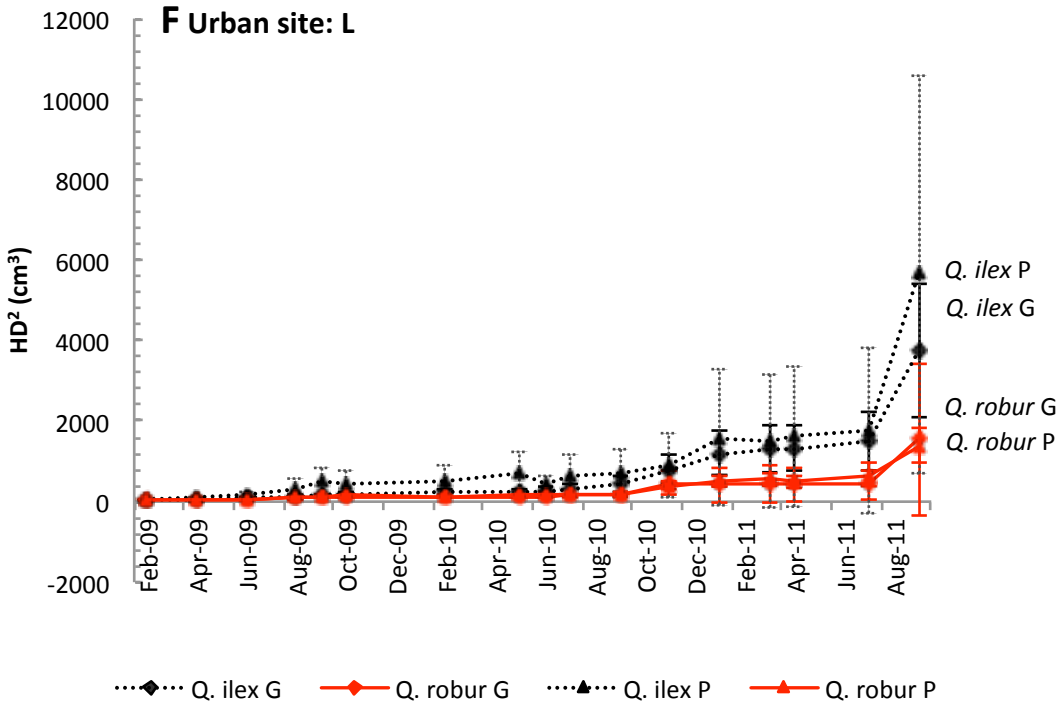


Figure E.6: Monthly growth for urban site L saplings over the 33 month growth period (means ± 95 % CI).

E.2 Relative growth rates (RGR).

The following figures are monthly and season relative growth rates for each species.

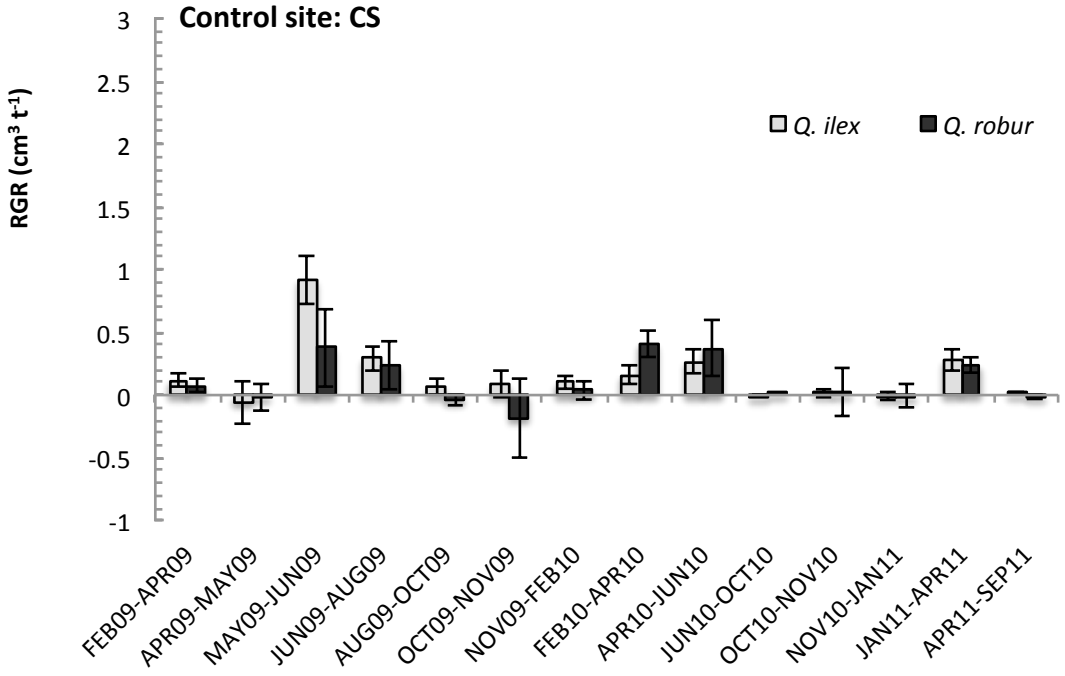


Figure E.7: Relative growth rates in control site potted saplings (means $\pm$ 95 % CI). Data shown is approximately two monthly but see axis for exact increments.

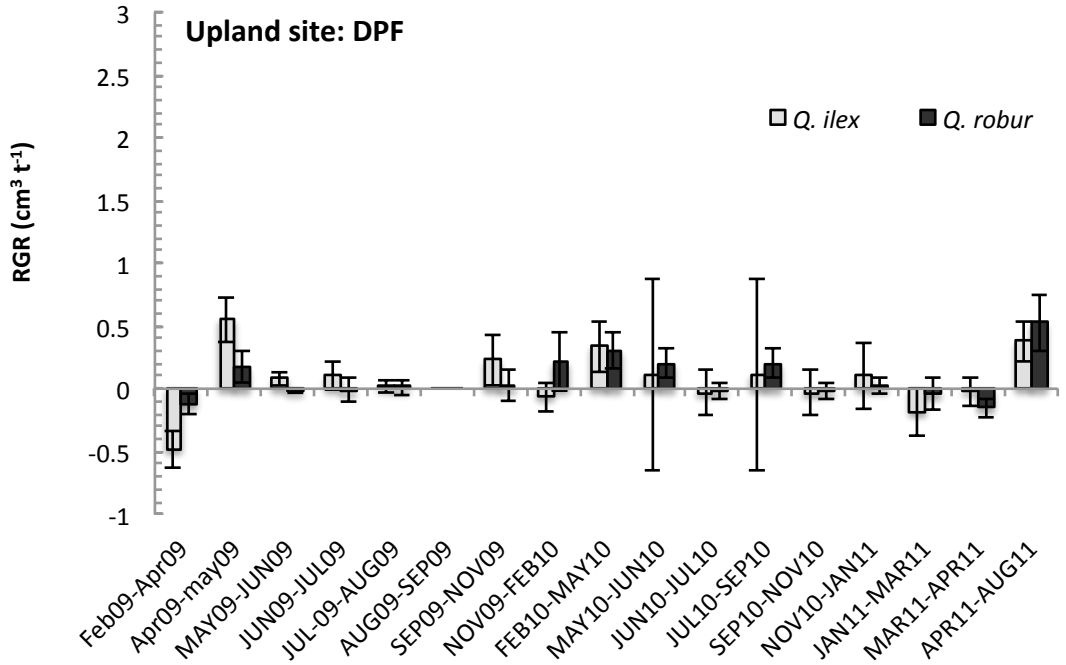


Figure E.8: Relative growth rates in upland site field grown saplings (means ± 95 % CI). Data shown is approximately two monthly but see axis for exact increments.

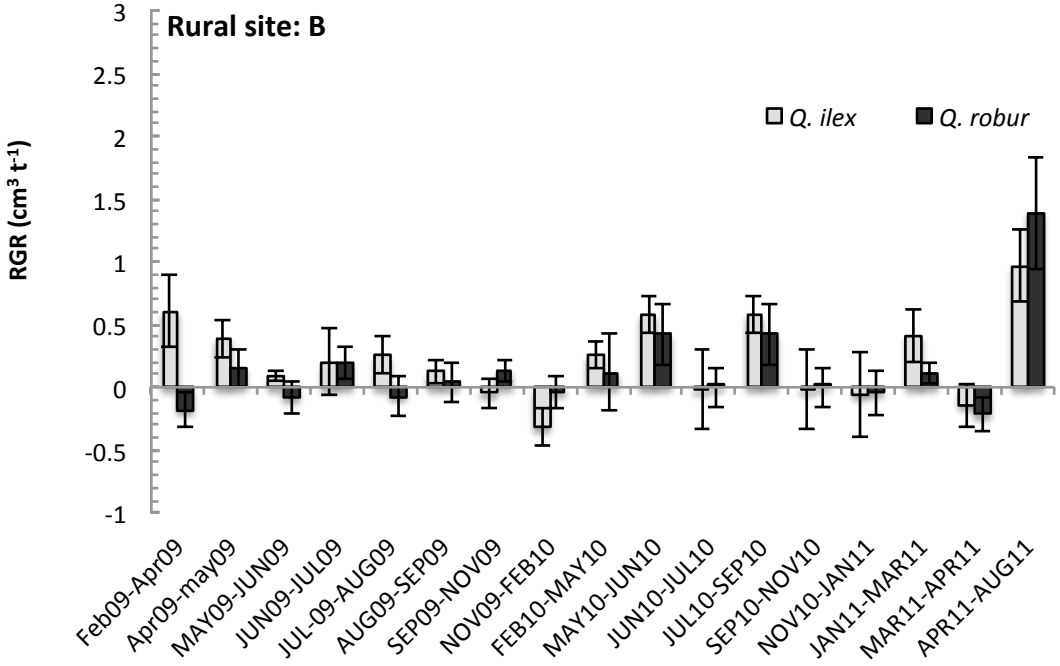


Figure E.9: Relative growth rates in rural site B, field grown saplings (means $\pm$ 95 % CI). Data shown is approximately two monthly but see axis for exact increments.

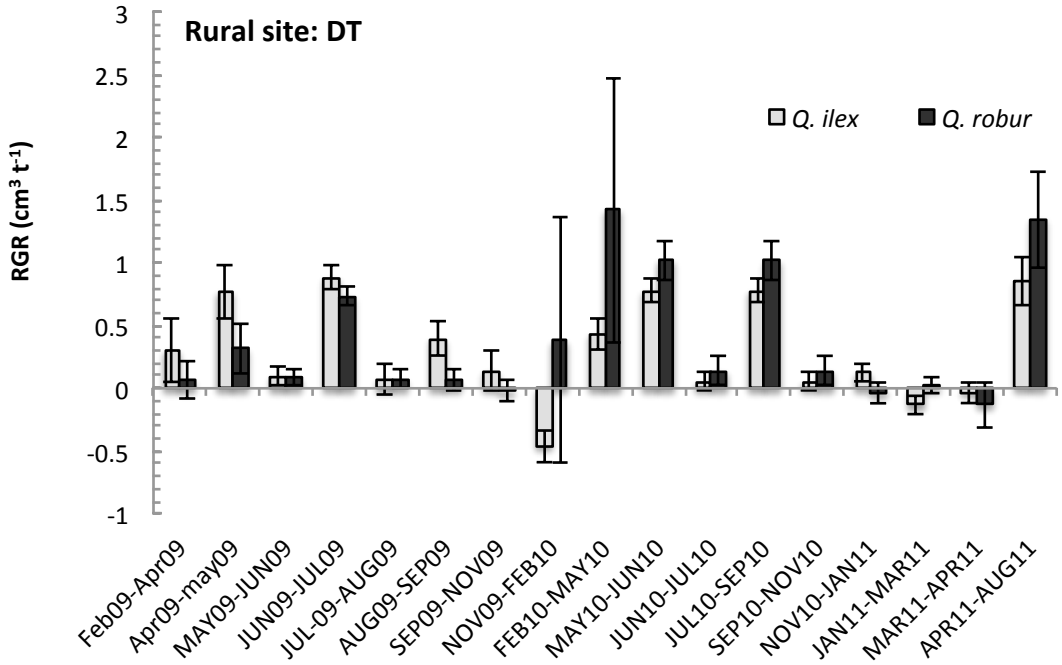


Figure E.10: Relative growth rates in rural site DT, field grown saplings (means ± 95 % CI). Data shown is approximately two monthly but see axis for exact increments.

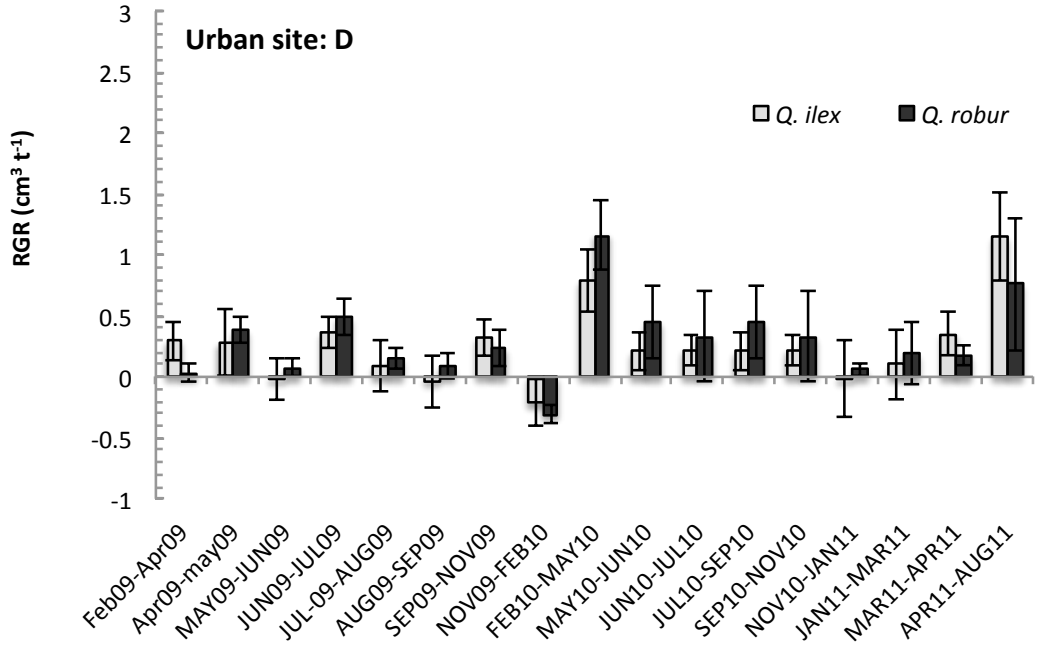


Figure E.11: Relative growth rates in upland site field grown saplings (means ± 95 % CI). Data shown is approximately two monthly but see axis for exact increments.

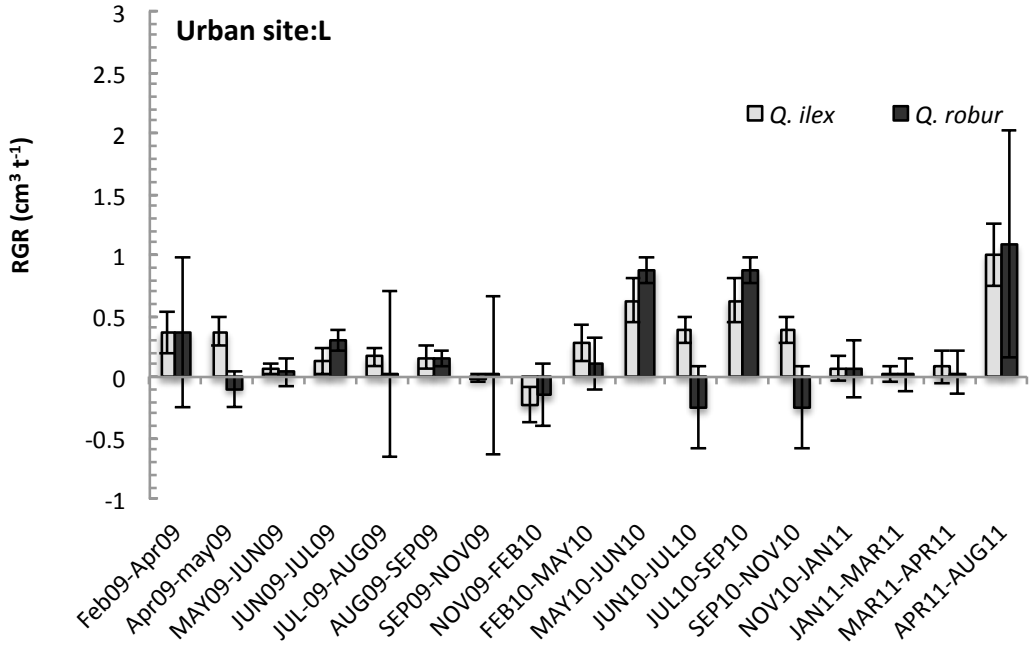


Figure E.12: Relative growth rates in urban site L field grown saplings (means $\pm$ 95 % CI). Data shown is approximately two monthly but see axis for exact increments.

E.3 Biomass descriptive statistics

The following two tables (tables E.1 and E.2) give descriptive statistics for the biomass variables given in chapter 6.

Table E.1: Final biomass and growth parameters for field grown saplings (Ground), August 2012 (g / tree).

Sapling type: GROUND			*Q. ilex*						*Q. robur*					
Growth parameter	Character	SITE	N	Mean	StDev	Min	Max	Skew	N	Mean	StDev	Min	Max	Skew
Leaf Biomass (g)	RURAL	B	10	58.33	21.24	18.6	95.85	-0.04	9	7.58	3.78	1.88	12.72	-0.3
		DT	10	139.7	72.7	34	247.1	0.01	10	61.6	34.4	21.3	129.7	0.86
	URBAN	D	8	78.9	31.4	40.7	127.9	0.21	10	31.03	18.69	4.12	60.78	-0.05
		L	11	55.4	36.6	20.8	128.9	1	3	12.77	8.59	4.6	21.72	0.41
	UPLAND	DPF	9	4.46	3.69	0.48	12.18	1.2	17	2.704	1.403	0.027	5.472	0.04
Branch Biomass (g)	RURAL	B	10	40.46	25.3	8.17	85.49	0.9	9	4.88	4.69	0.48	15.62	1.69
		DT	10	97.8	61	30.8	209	0.56	10	80	64.5	22.3	243.6	2.12
	URBAN	D	8	75.6	35	26.8	139.7	0.54	10	33.08	20.86	2.85	51.57	-0.73
		L	11	59.7	57.9	11.1	184.4	1.22	3	8.93	6.01	5.13	15.86	1.71
	UPLAND	DPF	9	1.964	1.263	0.118	4.156	0.37	17	0.6204	0.3406	0.174	1.222	0.44
Root Biomass (g)	RURAL	B	10	78.23	29.75	20.45	121.11	-0.54	9	22.1	13.69	7.73	42.96	0.44
		DT	10	111.2	38.9	62.9	161.6	-0.18	10	95.5	44	41	179.5	1.06
	URBAN	D	8	111.6	32.6	60.1	156.3	-0.68	10	81.1	53	16.5	191.9	0.72
		L	11	71.8	48	22.2	142.1	0.4	3	27.25	8.28	19.73	36.13	0.72
	UPLAND	DPF	9	8.83	3.53	3.63	13.05	-0.53	17	7.342	3.69	3.049	16.559	1.02
Stem Biomass (g)	RURAL	B	10	47.91	23.69	15.52	87.24	0.5	9	7.61	5.01	2.59	15.47	0.67
		DT	10	132.2	59.3	51.6	212.5	0.21	10	105.5	67	35.6	248.6	1.36
	URBAN	D	8	90.3	50.9	6.6	164.7	-0.52	10	78.4	53.1	8	167.9	0.4
		L	11	58.3	39.2	18.1	117.8	0.37	3	18.98	16.85	6.83	38.22	1.56
	UPLAND	DPF	9	3.912	1.616	1.817	7.01	0.56	17	3.296	1.707	1.017	6.813	0.65
Total Biomass (g)	RURAL	B	10	224.9	94.7	62.7	378.4	0.24	9	42.18	21.59	13.15	69.34	-0.07
		DT	10	480.9	183	187.9	814.8	0.09	10	327.8	186.5	116.1	722.2	1.34
	URBAN	D	8	363.1	155.9	138.5	624.6	-0.03	10	223.6	138.8	33.8	468	0.11
		L	11	239.4	171.5	57.4	567	0.77	3	66.6	37	37	108.1	1.29
	UPLAND	DPF	9	19.17	8.61	7.31	31.44	-0.19	17	13.96	5.83	5.56	26.73	0.34
Aboveground Biomass (g)	RURAL	B	10	146.7	67.8	42.3	268.6	0.58	9	20.08	12.14	4.95	40.59	0.41
		DT	10	369.6	154.2	116.4	668.6	0.2	10	232.4	146.4	75.1	542.8	1.31
	URBAN	D	8	251.5	124.8	78.4	468.3	0.18	10	142.5	90	15.1	276.1	-0.08
		L	11	169.8	124.7	52.9	431.1	1.05	3	39.4	28.8	17.2	71.9	1.41
	UPLAND	DPF	9	10.33	5.59	2.41	19.93	0.34	17	6.621	3.03	2.514	11.102	0.13
mean HD^2 (cm^3)	RURAL	B	10	2526	1280	893	4997	0.73	9	502	368	117	1120	0.61
		DT	10	9413	4583	3216	17846	0.59	10	8023	7410	1628	26664	2.06
	URBAN	D	8	6616	3119	1863	11983	0.39	10	8237	6598	464	21139	0.79
		L	11	3769	2844	1062	8813	1.01	3	1549	1672	369	3463	1.59
	UPLAND	DPF	9	483	910	62	2902	2.97	17	186.6	114.8	57.5	409.4	0.77

Table E.2: Final biomass and growth parameters for pot grown saplings (POTs), August 2012 (g / tree).

Sapling type: POTS			*Q. ilex*						*Q. robur*					
Variable		SITE	N	Mean	StDev	Min	Max	Skew	N	Mean	StDev	Min	Max	Skew
Leaf Biomass (g)	RURAL	B	9	85.7	57.5	18.9	176	0.28	10	15.74	7.72	3.13	28.87	-0.25
		DT	7	97.5	101.2	15.1	257.3	0.71	9	29.74	25.64	10.1	90.43	2
	URBAN	D	6	129.1	70.3	39.4	228.7	0.11	10	27.48	14.13	9.13	56.6	0.92
		L	4	78.9	65.2	16	166.8	0.96	10	14.36	6.72	6.42	27.12	0.76
	UPLAND	DPF	8	10.63	19.97	1.06	59.64	2.73	12	13.62	15.12	0.39	53.07	2.09
	CONTROL site	C	8	52.17	12.34	36.63	70.2	-0.03	7	12.86	6.29	5.08	21.27	0.15
Branch Biomass (g)														
	RURAL	B	9	57.3	41.4	10.7	115.2	0.25	10	7.74	3.88	2.56	12.96	-0.24
		DT	7	102.4	123	8.8	323.9	1.08	9	38.5	32.2	10.6	114.4	1.93
	URBAN	D	6	116.5	71.1	19.8	212.9	0.02	10	31.81	28.69	9.6	99.93	1.88
		L	4	87.4	93.2	15.5	215.9	1.21	10	10.41	3.76	5.43	17.49	0.97
	UPLAND	DPF	8	7.69	12.66	0.88	38.4	2.62	12	9.25	12.41	0.17	40.08	2.01
Root Biomass (g)	CONTROL site	C	8	33.07	8.09	18.69	44.54	-0.62	7	17.7	33	1.2	92	2.58
	RURAL	B	9	128.2	78.5	47.3	234.7	0.27	10	37.71	14.39	11.89	56.43	-0.96
		DT	7	108.2	109.3	20	283.5	1.19	9	78.1	74.2	33.4	267.4	2.56
	URBAN	D	6	161.7	93.1	40.5	246.1	-0.61	10	98.5	52.6	58.6	232.5	2.17
		L	4	157.4	99.7	39.2	279	0.09	10	60.18	29.62	37.15	128.02	1.63
Stem Biomass (g)	UPLAND	DPF	8	25.3	31.8	5.5	100.9	2.43	12	36.6	37.6	1.7	138.3	2.08
	CONTROL site	C	8	91.04	20.77	62.31	119.79	-0.28	7	35.01	13.17	17.35	50.34	-0.58
	RURAL	B	9	54.7	36.3	16.3	106.4	0.49	10	12.58	7.88	1.74	25.99	0.27
		DT	7	106.9	111.9	13.8	278.1	0.64	9	62.2	63.9	7.1	208.8	1.71
	URBAN	D	6	114.3	52.2	33.2	157.8	-1.06	10	48.47	23.19	14.8	93.03	0.5
Total Biomass (g)		L	4	85.9	77.4	14.5	173.4	0.26	10	16.92	11.04	7.4	35.32	0.88
	UPLAND	DPF	8	9.12	14.17	1.88	43.97	2.76	12	16.69	17.84	1.11	65.2	2.16
	CONTROL site	C	8	43.28	10.45	30.93	59.25	0.22	7	7.08	3.11	2.98	12.3	0.68
	RURAL	B	9	320.4	172.7	108.9	606.6	0.13	10	73.78	29.96	21.74	115.77	-0.99
		DT	7	406	391	59	862	0.38	9	205.5	185.7	54.9	666.9	2.28
Aboveground Biomass (g)	URBAN	D	6	519	277	134	801	-0.54	10	203.6	114.2	93.1	482.1	1.8
		L	4	426	357	85	900	0.87	10	102.4	37.4	65.7	164	0.68
	UPLAND	DPF	8	53.4	78	12	242.9	2.63	12	76.1	80.5	3.4	296.6	2.16
	CONTROL site	C	8	218.7	46.5	151.1	269	-0.61	7	72.6	41.2	29.5	150.5	1.04
	RURAL	B	9	192.2	130.7	49.8	373.4	0.4	10	36.07	18.22	7.71	63.92	-0.27
mean HD^2 (cm^3)		DT	7	298	318	39	800	0.68	9	127.4	116.6	21.5	399.6	1.84
	URBAN	D	6	357.6	187.3	78.7	555.1	-0.56	10	105.1	63	34.6	249.6	1.43
		L	4	268	261	46	621	1.03	10	42.2	19.08	26.29	70.65	0.84
	UPLAND	DPF	8	28.1	46.5	6.3	142	2.73	12	39.6	43.9	1.7	158.3	2.17
	CONTROL site	C	8	127.7	29.9	88.8	167	-0.31	7	37.6	37.2	12	117.9	2.17

Appendix F

Scattergraphs

F.1 Scatter graphs for biomass and stem volume relationships.

The following graphs give scatter relationships between biomass and stem volume for all sites and combinations and clearly show the linear relationship between the two variables. Sites where saplings have experienced stress such as *Q. robur* at the least fertile and drier urban site L and *Q. ilex* growing at the upland site DPF have weaker relationship than those saplings growing at the more fertile sites where water availability is greater (see figure F.2).

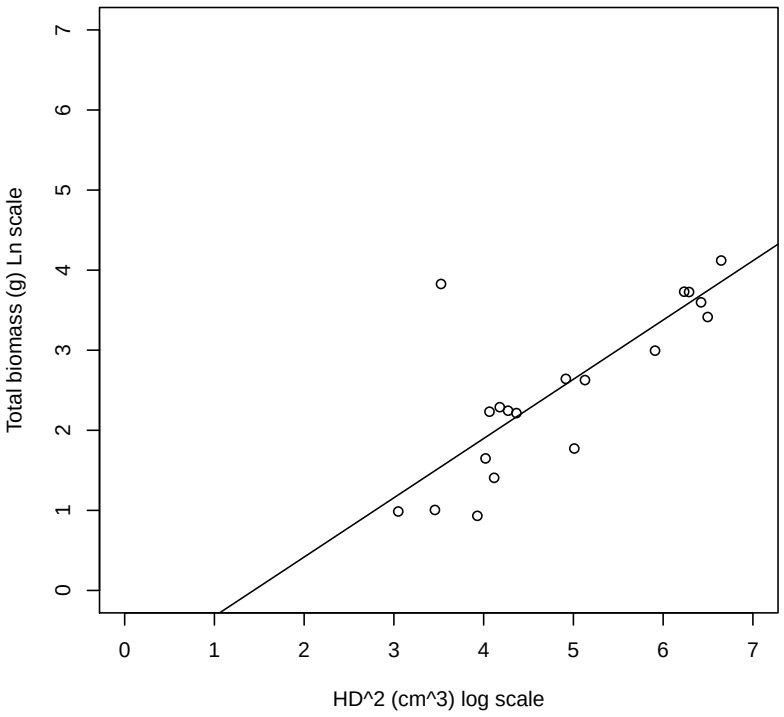


Figure F.1: Relationship between total biomass TB and stem volume HD^2, initial $harvest_1$.

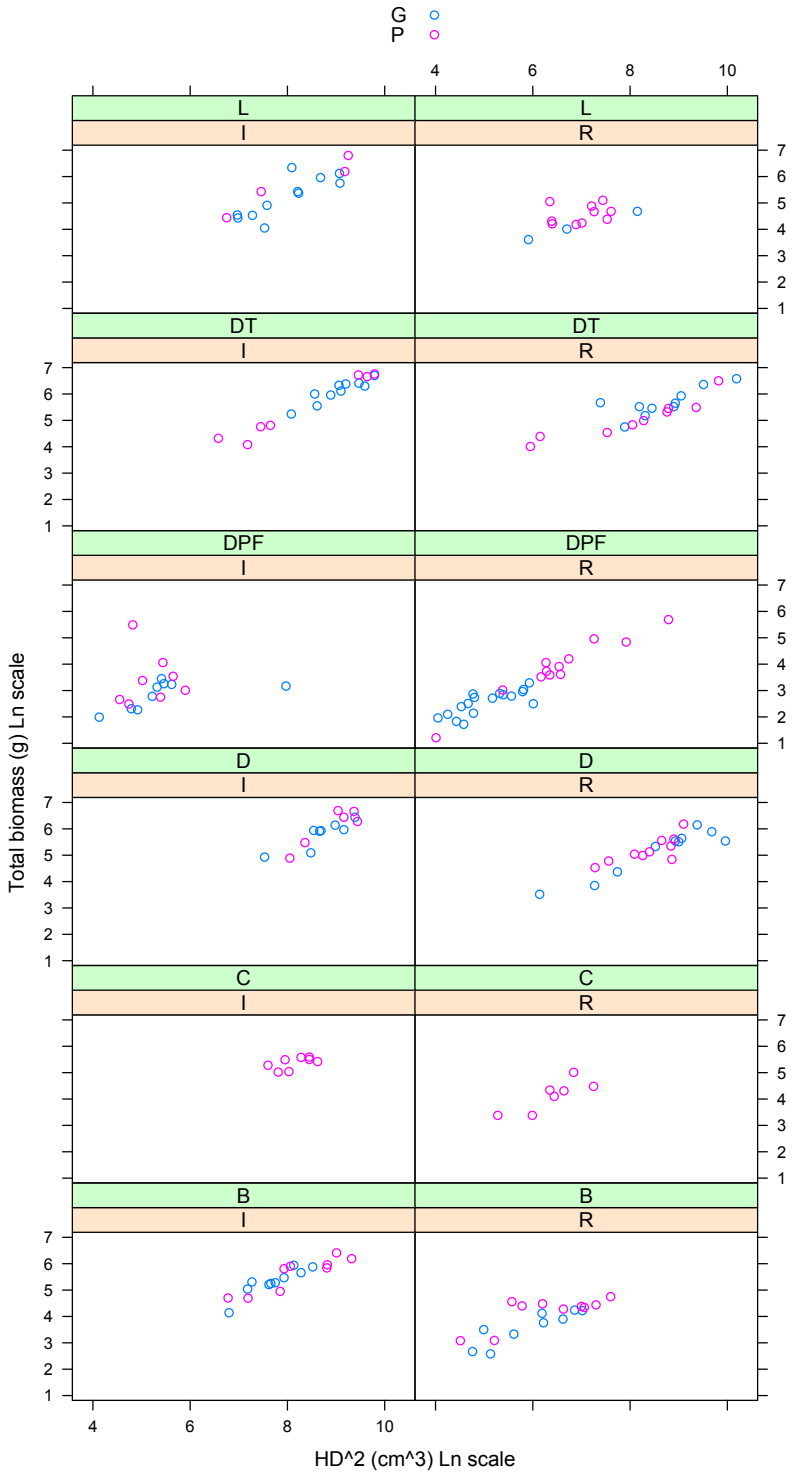


Figure F.2: Relationship between total biomass TB and stem volume HD^2, grouping for pots (P) versus field grown (G) saplings ('I' = *Q. ilex* and 'R' = *Q. robur*, sites: 'C' = Control Site, 'DPF' = Upland, rural sites, 'B' = Broadley, 'DT' = Dartington, and urban sites, 'D' = Digginit and 'L' = laira)

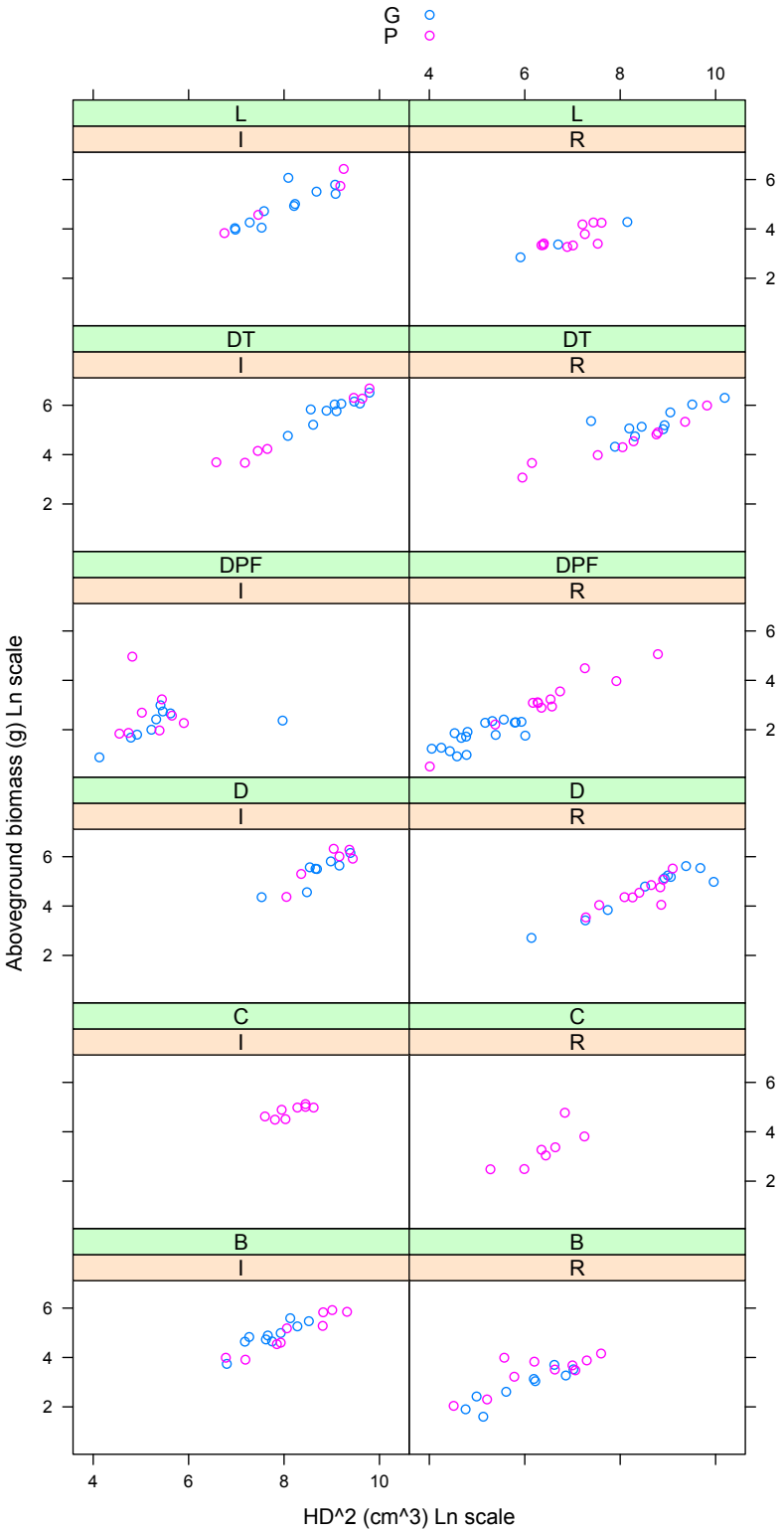


Figure F.3: Relationship between aboveground biomass *AGB* and stem volume HD^2, grouping for pots (P) versus field grown (G) saplings ('I' = *Q. ilex* and 'R' = *Q. robur*, sites: 'C' = Control Site, 'DPF' = Upland, rural sites, 'B' = Broadley, 'DT' = Dartington, and urban sites, 'D' = Digginit and 'L' = laira)

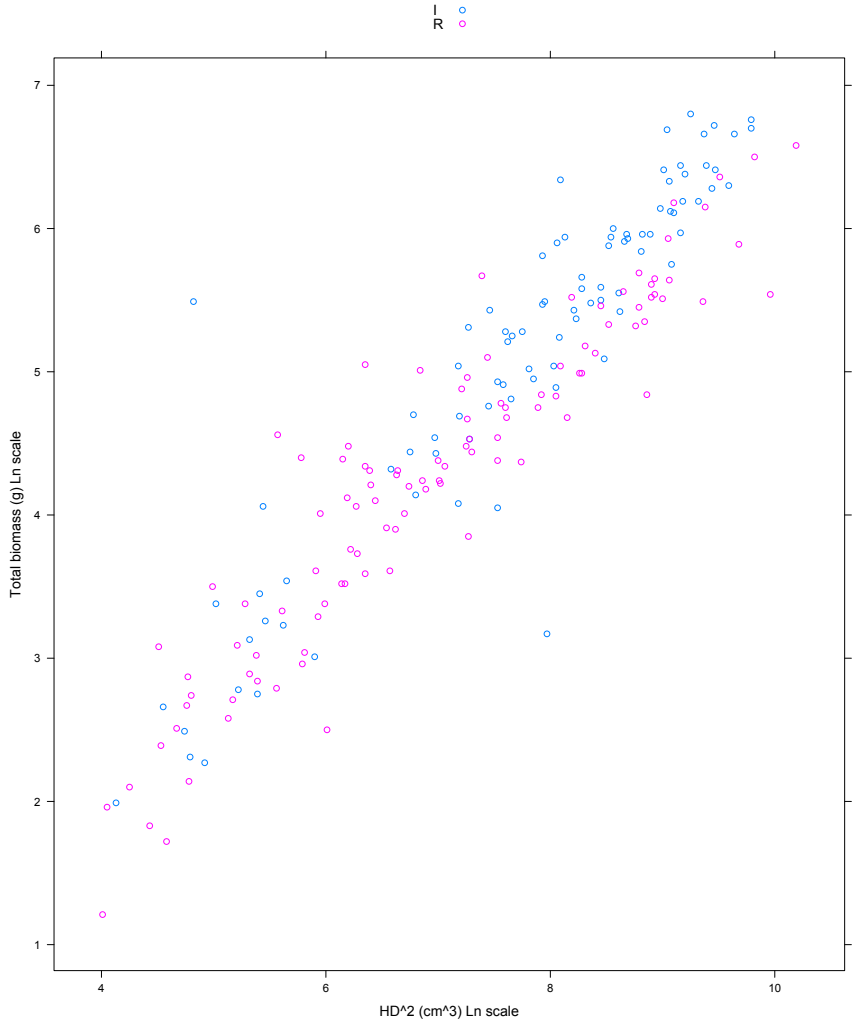


Figure F.4: Relationship between total biomass TB and stem volume HD^2 on *ln* scale. Grouping for pots (P) versus field grown (G) saplings ('I' = *Q. ilex* and 'R' = *Q. robur*, sites: 'C' = Control Site, 'DPF' = Upland, rural sites, 'B' = Broadley, 'DT' = Dartington, and urban sites, 'D' = Digginit and 'L' = laira)

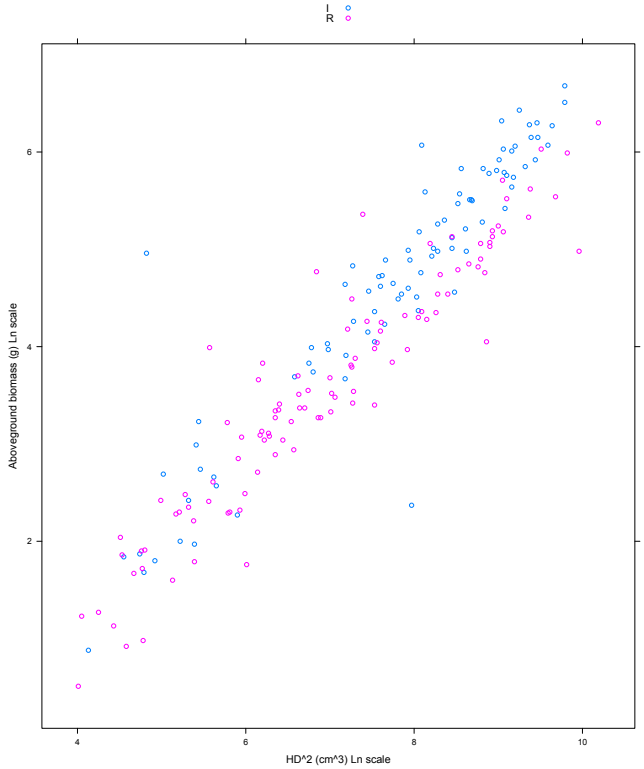


Figure F.5: Aboveground biomass *AGB* and stem volume HD^2 on a *ln* scale. Grouping for pots (P) versus field grown (G) saplings ('I' = *Q. ilex* and 'R' = *Q. robur*, sites: 'C' = Control Site, 'DPF' = Upland, rural sites, 'B' = Broadley, 'DT' = Dartington, and urban sites, 'D' = Digginit and 'L' = laira)

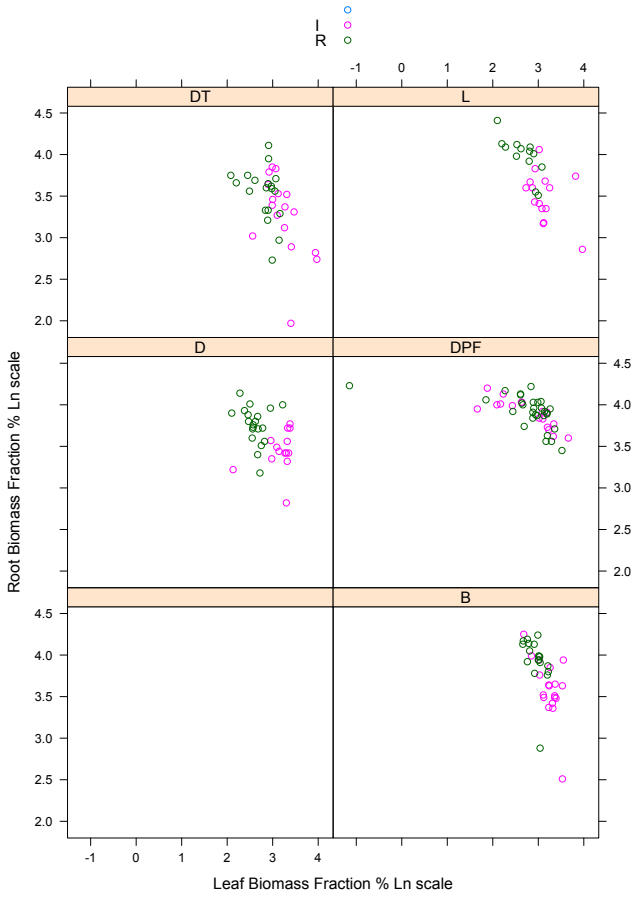


Figure F.6: Relationship between leaf weight fraction LWFt and root weight fraction RWFt allocation patterns for *Q. ilex* 'I' and *Q. robur* 'R' at different sites

Appendix G

Information on Light levels and filter densities for Light Response Curves

Light-unit + 0.1 + 0.2 + 0.3 ND Filters: P.A.R. $\approx$ 100 mols m^2/s.

Light-unit + 0.1 + 0.2 ND Filters: P.A.R. $\approx$ 180 mols m^2/s.

Light-unit + 0.1 + 0.3 ND Filters: P.A.R. $\approx$ 225 mols m^2/s.

Light-unit + 0.3 ND Filters: P.A.R. $\approx$ 250 mols m^2/s.

Light-unit + 0.2 ND Filters: P.A.R. $\approx$ 340 mols m^2/s.

Light-unit + 0.1 ND Filters: P.A.R. $\approx$ 410 mols m^2/s.

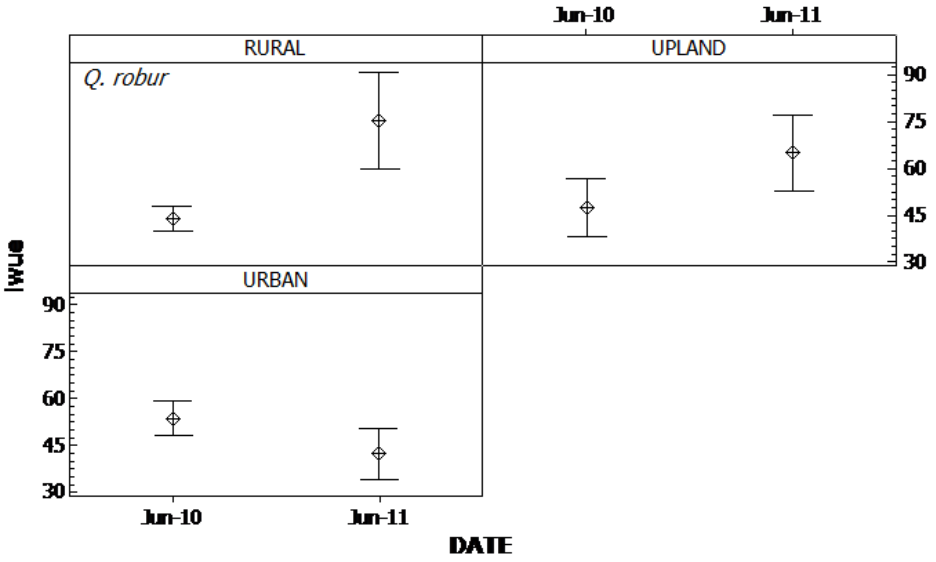
Light Unit without ND filter: P.A.R. $\approx$ 500 P.A.R. mols m^2/s.

Natural Light: 800-1800 P.A.R. (depending on weather).

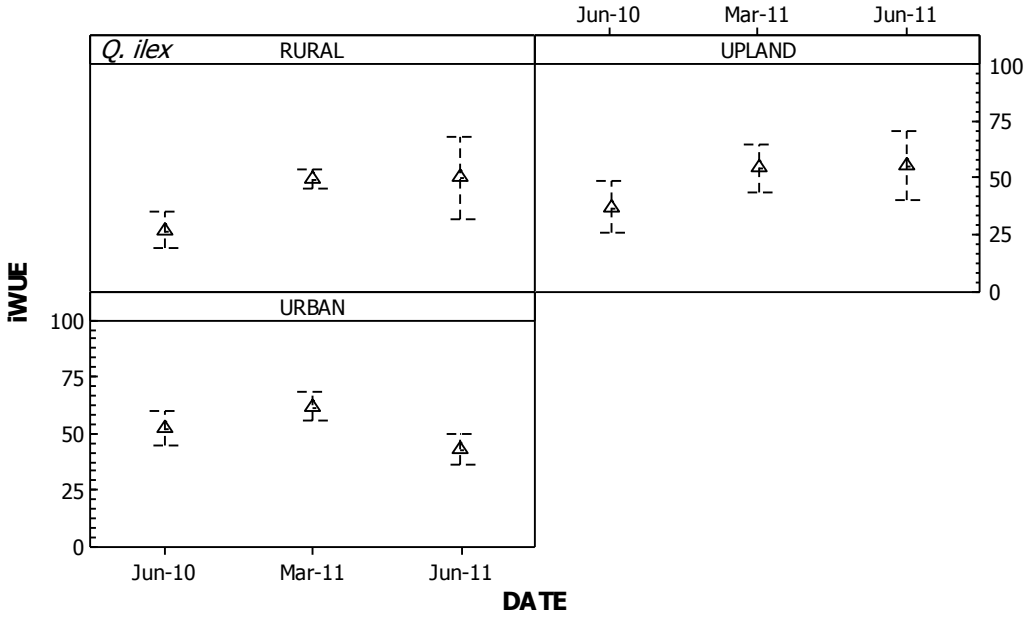
Appendix H

Mature trees: extra graphs.

The following figures show differences between dates for both *Q. robur* and *Q. ilex* for photosyntheitc variables, no data for leaf trait *SLA*, N_{leaf} and C_{leaf} shown.

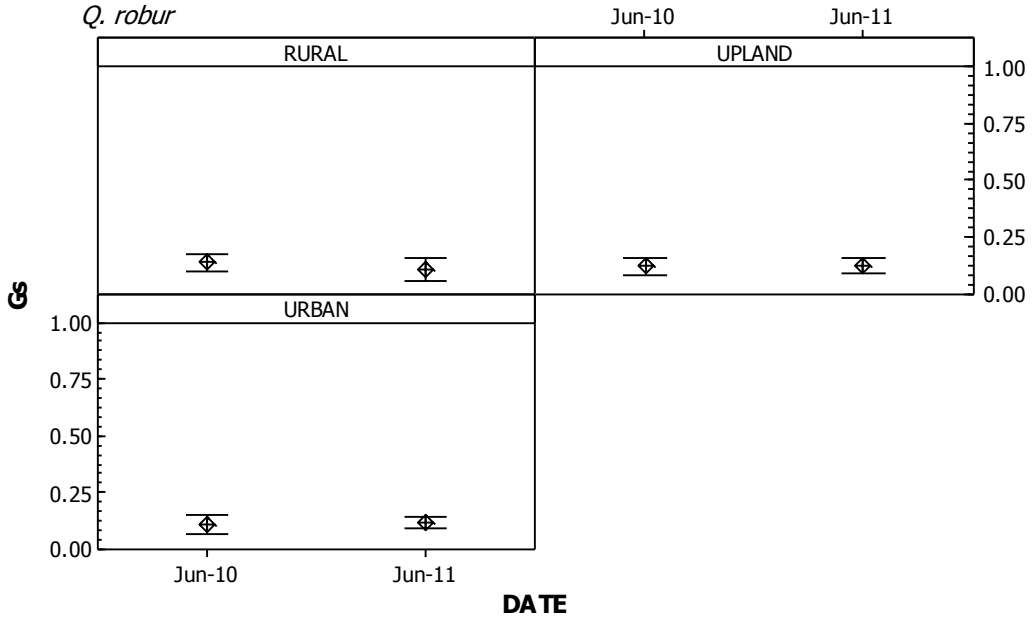


(a) Q. robur iWUE, Date

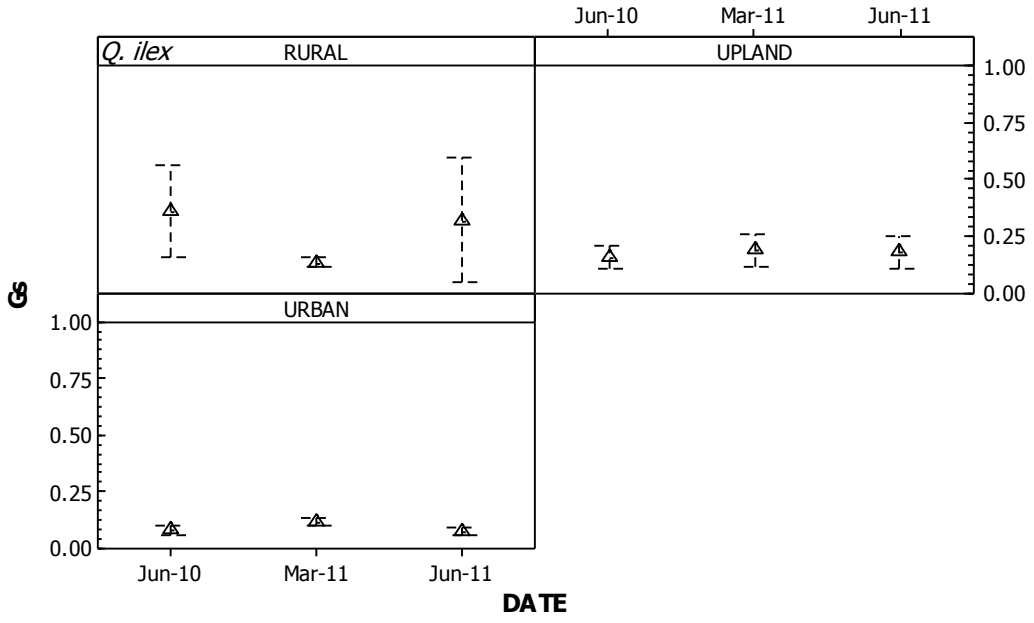


(b) Q. ilex iWUE, Date

Figure H.1: Differences in canopy level intrinsic water use efficiency (iWUE) between species and dates (means ± 95 % CI).

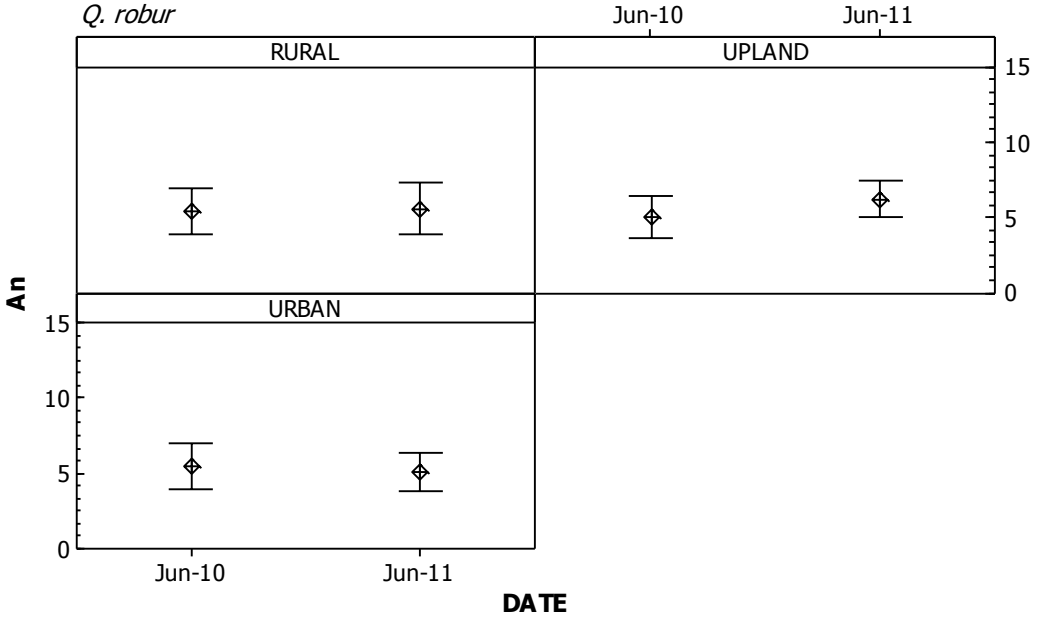


(a) Q. robur Gs, Date

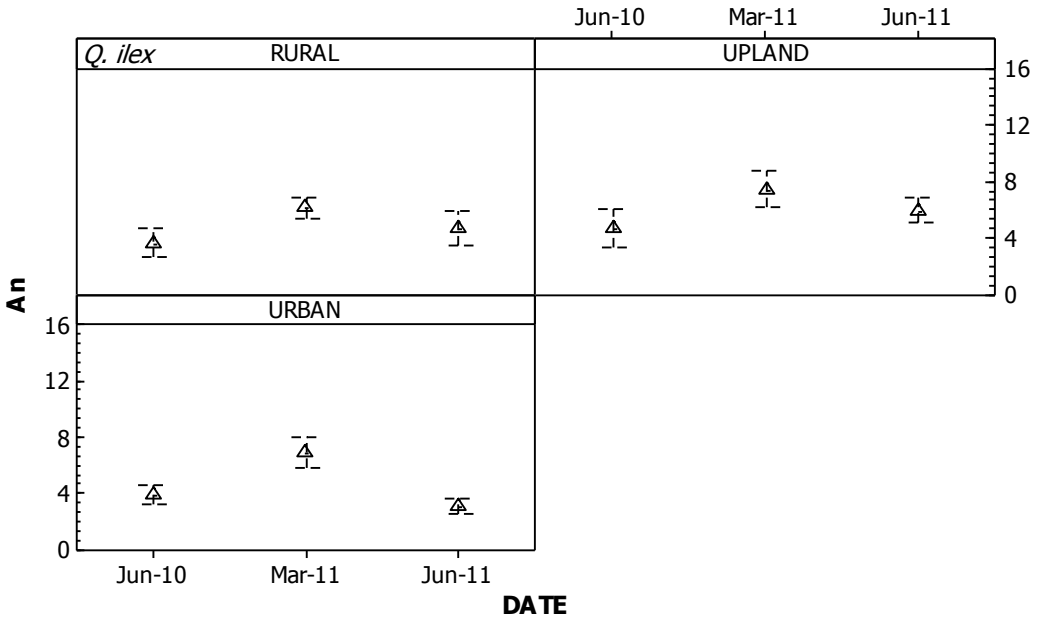


(b) Q. ilex Gs, Date

Figure H.2: Canopy *Gs*:Differences between dates (means ± 95 % CI).

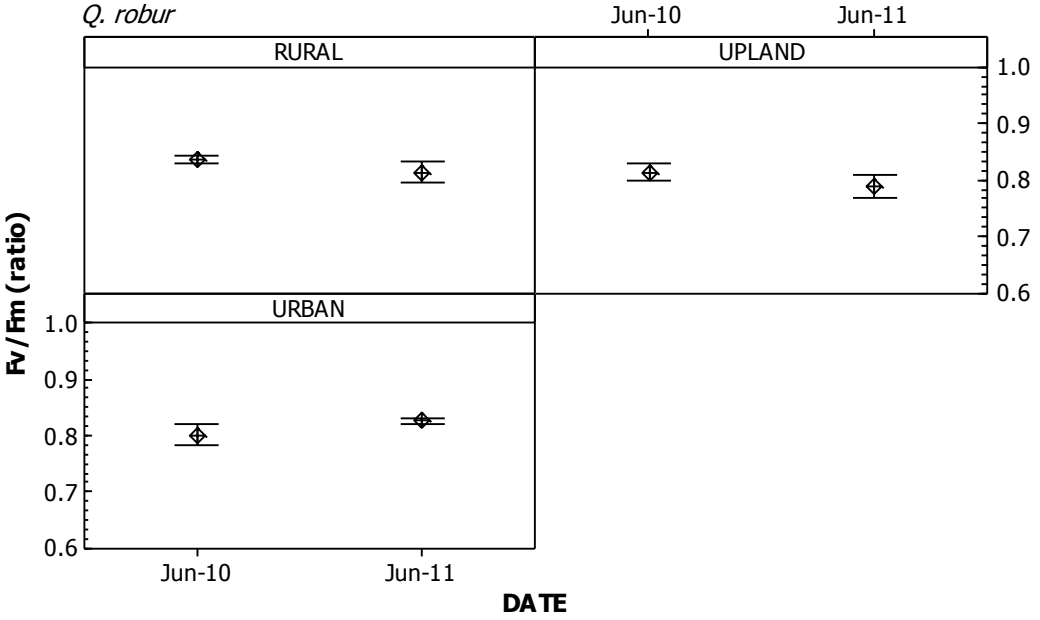


(a) Q. robur A_n, Date

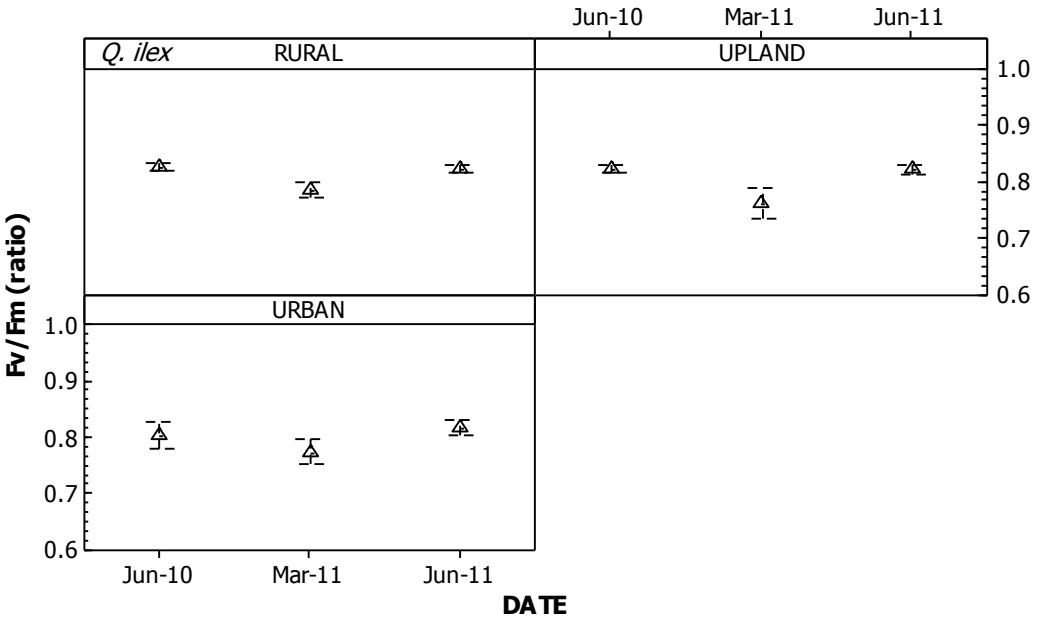


(b) Q. ilex A_n, Date

Figure H.3: Canopy A_n:Differences between dates (means $\pm$ 95 % CI).

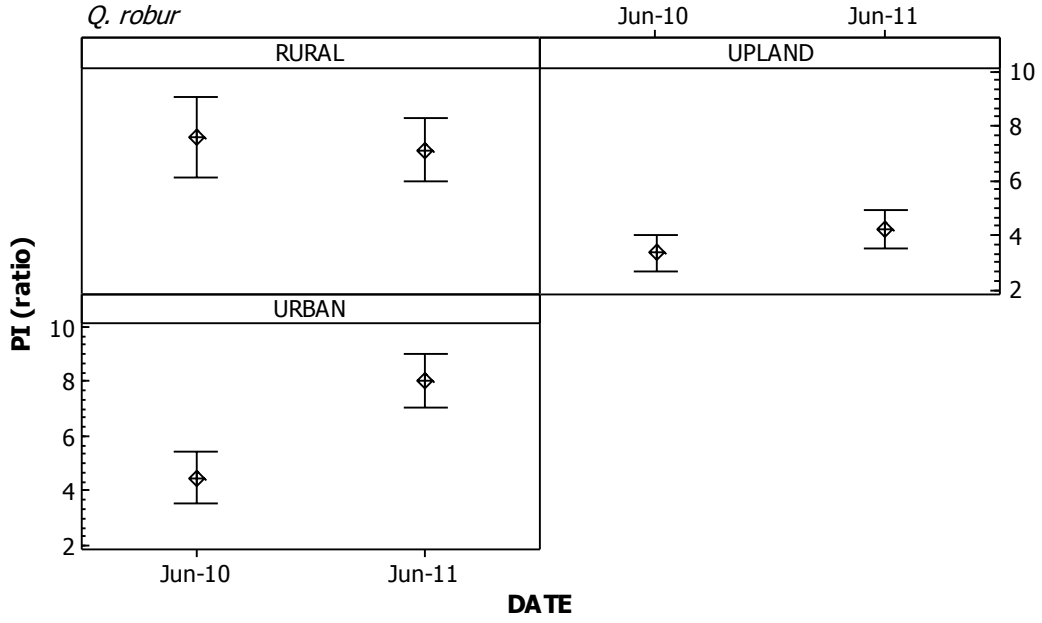


(a) Q. robur F_v/F_m, Date

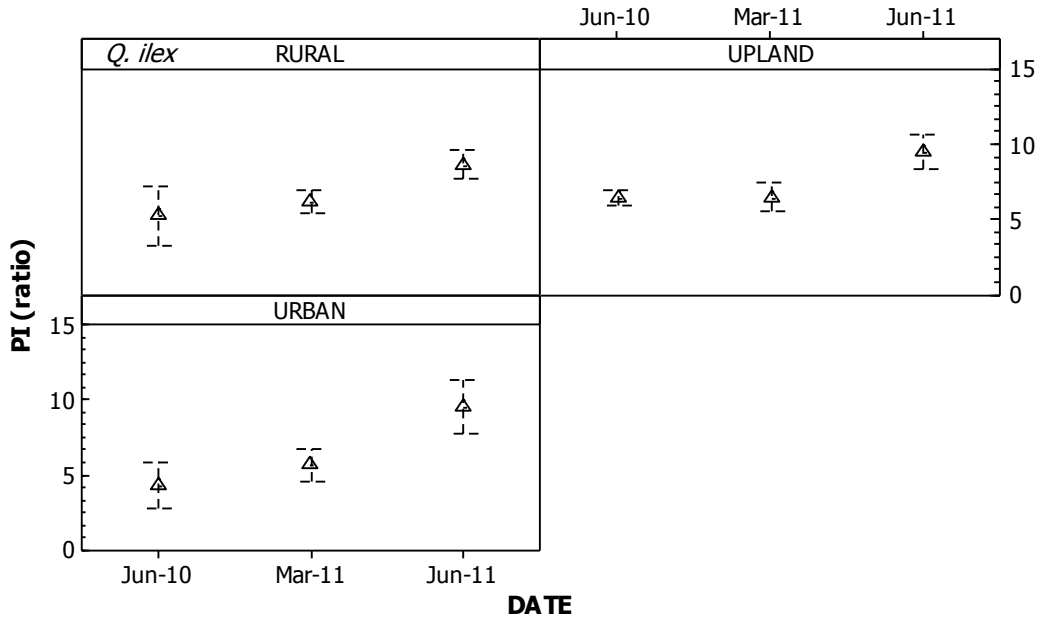


(b) Q. ilex F_v/F_m, Date

Figure H.4: Canopy F_v/F_m:Differences between dates (means $\pm$ 95 % CI).



(a) Q. robur PI, Date



(b) Q. ilex PI, Date

Figure H.5: Canopy *PI*:Differences between dates (means ± 95 % CI).

Glossary.

$\mu g\ m^3$ micrograms per cubic metre

A_n Net photosynthetic rate measured in $\mu\ mol\ m^{-2}\ s^{-1}$

A_{max} maximum photosynthetic rate,$\mu mol\ m^{-2}\ s^{-1}$.

C atmospheric Carbon

CF Correction factor for the back transformation of a logged power function

GHG Greenhouse gas; a gas that can absorb and re-emit infrared radiation, thus adding to the greenhouse effect (warming of the lower atmosphere as GHG re-emit infrared radiation back towards the earths surface

Gs stomatal conductance of CO_2 (Gs) measured in $mol\ m^{-2}\ s^{-1}$

$iWUE$ Intrinsic water use efficiency, measured as A_n/Gs in $\mu mol\ CO_2$ / $mol\ H_2O^{-1}$

LAR Leaf Area Ratio, ($m^2 g^{-1}$)

LMA leaf mass per unit leaf area, measured as 1/SLA $mg\ cm^{-2}$

Log_e or Ln Natural log of a number is the power to which e would have to be raised to get that number where $e \approx 2.7182$

LWR Leaf Weight Ratio, gg^{-1}

mg One milligram me = 0.001g.

RGR mean relative growth rate, modeled as the difference between mean of weight at harvest at time 2 ($_2t$) minus mean of weight of harvest at time 1 ($_1t$) divided by the difference between $_2t$ and $_1t$ and measured in $g\ day^{-1}$

AGB above ground biomass

AK available potassium in soils

Anisohydric behaviour. The'risk taking' action of keeping stomata open to increase assimilation rates despite lowered water availability and leaf water potential (see (e.g., Sade et al. 2012))

Aoil Auger. Special hand drill for removal of soil layers

AP available phosphate in soils

B Factor SITE: Broadley rural allotment, near South Brent Devon

BEF Biomass expansion factor: additive allometric equations that use the relationship between SW and different biomass fractions (e.g. branch, root, foliage) to model TB and AGB from DBH and H parameters

BRP A combination of factors: SITE, SPECIES and CONDITION

CS Factor SITE: Control site, poly-tunnel situated in SE Cornwall

CYM Current year mature leaf

D Factor SITE: Digginit Community allotment,urban site located in Plymouth

DPF Factor SITE: Dartmoor Prison farm, Upland site

DT Factor SITE: Dartington, rural allotment near Totnes, Devon

E Mean transpiration rate (E measured in $mmol\ m^{-2}\ s^{-1}$

Flow-rate Rate at which $\mathbf{CO_2}$ is passed through the leaf chamber of the IRGA

G Factor CONDITION; field grown saplings

g grammes

GLM General Linear Model, using least squares regression and allowing multiple factors to be analysed against a dependent variable and assuming homogeneity of variances and normal distribution

GtC Gigatonnes of atmospheric Carbon

I Factor SPECIES *Quercus ilex* subsp. *ilex*, common name: Holly or Holm oak

IRGA Infra-red Gas Analyser

L Factor SITE: Laira, urban allotment, located in Plymouth

LA Leaf area, cm^2

LAR Leaf area ratio: Leaf area/ dry plant mass

MAT Mean Annual Temperatures.

mg Milligrammes in weight

N mineral nitrogen, insoluble nitrogen in soils

NL Newly formed, immature current year leaf

Oceanic warm temperate, fully humid with warm (not hot) summers, mild winters and evenly distributed precipitation

Ontogenetic Relating to growth and developmental stages in an individuals life

P Factor CONDITION; Saplings planted in pots

P.A.R. Photosynthetically active radiation; the light that plants can absorb in order to carry out the functions of photosynthesis, from the electro magnetic spectrum, visible light consisting of wavelengths between 400-700 nanometers (nm)

pH Acidity/alkalinity of soils

Phytaphagus e.g. A phytaphagus insect feeds off plants

Plasticity The ability of a species to cope with changes in its normal environmental conditions; a more plastic species being able to tolerate changes better than one with less plasticity (this may or may not be subject to selection)

PNUE Photosynthetic Nitrogen Use Efficiency, A_n / N_{leaf} ratio

ppm Parts per million

R Factor SPECIES *Quercus robur*, common name: pedunculate or English oak

Root/Shoot ratio. Ratio of root mass divided by stem and/or total aboveground biomass

Sapling. Young tree. In this research between one and four years in age

SLA Specific leaf area; the ratio of leaf area to leaf dry weight $cm^2 g^{-1}$

Specific gravity. Specific wood gravity determines how much of a trees structural growth is attributed towards strength and support (Williamson and Wiemann 2010) and is determined by dividing oven dry mass (ODM) by water displaced by the same sample when green, or measured as density, as ODM divided by green volume ($g^{-1} cm^3$) (Muller-Landau 2004)

TB total biomass

TLA total leaf area

TN Total nitrogen content in soils

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ADC Bioscientific (2007), 'Lci portable infra-red gas analyser (irga)'.

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