The Ecological Effects of Ozone Exposure on Upland Semi-Natural Vegetation of the Yorkshire Dales National Park

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Abstract:

Tropospheric ozone is a pollutant which has been shown to cause significant effects on crop species, but its impact on semi-natural vegetation remains uncertain. This study aimed to evaluate the impact of ozone on communities of conservation importance within one area of the UK - the Yorkshire Dales National Park; an upland area was selected because ozone exposure in upland areas is predicted to be higher and increase more rapidly than in lowland areas. Individual plant species and woodland mesocosms taken from the study area were exposed to ozone under controlled environmental conditions. The results suggest that species and ecotypes of limestone communities were relatively insensitive to ozone, but that characteristic species of woodland ground flora communities could be adversely affected. The study also identified subtle morphological changes in grassland species and a greater impact on root compared with shoot biomass. The results highlight adverse effects on ecological fitness caused by ozone exposure and the results are placed in the wider context of woodland ecology.

Keywords: ozone, woodlands, uplands, mesocosms, conservation, ground flora.

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Authors Declaration

I declare that the research outlined here has not been presented before and is solely my own research.

1. Chapter 1: Introduction

1.1 Ozone in the Atmosphere

Ozone is naturally found in small concentration in the Earth's stratosphere and troposphere. It is a molecule comprising of three oxygen atoms which is unstable and highly reactive. In the upper layers of the atmosphere, ozone has a beneficial effect by absorbing harmful ultra violet radiation, reducing the levels that reach the Earth's surface. Ground level ozone, in contrast, is considered a serious pollutant that affects human health, reduces crop yields and damages natural ecosystems where present in sufficient concentrations (Ashmore and Bell, 1991). It was in the 1950's that ozone was first identified as a major component of the Los Angeles' smog. In Europe, ozone is now thought to be one of the most important air pollutants (EEA, 2005).

There are two major sources of tropospheric and ground-level ozone. These are natural mixing and air movement bringing down ozone from the stratosphere, and the photochemical production of ozone within the troposphere. Ozone is produced by the photochemical reactions of nitrogen oxides (NO_x, composed of NO + NO_2) and volatile organic compounds (VOC's). A wide range of VOC's occur naturally from chemicals manufactured by plants. The source of much of the NOx and VOC's emissions is now anthropogenic, through, for example, the burning of fossil fuels in power stations and cars.

The lifetime of ozone in the troposphere varies with altitude, and ranges from 1-2 days in the boundary layer close to the ground, to several weeks in the upper troposphere. Lifetime is determined by the removal processes (the sinks). The

most important of these are chemical removal within the troposphere, and removal at the surface, by terrestrial vegetation, soil and water surfaces by a process referred to as dry deposition (Coyle *et al.*; 2003a).

Ozone photodissociates to from energentic oxygen molecules which react with water vapour to produce peroxyl and hydroxyl radicals; this process is the main process for chemical destruction of ozone. However, if organic molecules are present, these radicals react to form HO_2 and other organic-peroxy radicals, which are able to react with NO to generate NO_2 for further production of ozone (Hayles, 1996).

The surface deposition flux includes uptake by vegetation. The long life-time of the precursors of ozone in the troposphere (mean 22 (±2) days; Stevenson *et al.*, 2006) means that they can be transported by wind over 1000 km – 2000 km per day.

The pattern of ozone deposition to vegetation varies over a 24 hr-period; typical diurnal cycles in ozone concentrations have a mid-afternoon peak and night time minimum (Coyle *et al*; 2003a). Ozone in lowland Britain is usually absent or at low concentrations at night, but during ozone episodes, ozone in upland areas can be present at significant concentrations at night, albeit at lower levels than during the day. Typically, seasonal peaks in ozone pollution coincide with hot sunny weather and occur during the summer months; this has had impacts for vegetation as this is the peak of photosynthetic activity. Damage to vegetation is primarily caused by the uptake of ozone through stomata (Fowler *et al*; 1998); therefore

high values of stomatal conductance are associated with high values of ozone flux into plants.

1.1.2 Ozone and greenhouse gases

There are important links between many of the traditional air pollutants of importance and greenhouse gases and global warming. Ozone itself, is a 'greenhouse' gas, as it both absorbs longer-wave radiation from the Earth's surface (Ashmore & Bell, 1991) and indirectly influences the longevity of other greenhouse gases such as methane (Fiore *et al.*, 2002). Ozone therefore has a direct effect on climate through radiative forcing, and its impact as an anthropogenic greenhouse gas is third only to carbon dioxide and methane (European Commission, 2003). Recent studies also suggest that the indirect effect of ozone in causing radiative forcing, through reductions in the terrestrial carbon sink, may also be significant (Sitch *et al.*, 2007).

Methane, carbon monoxide, and NOx, at tropospheric levels, are all involved in the formation of O_3 (Bytnerowicz *et al.*, 2007). Climate change is a potentially important driver of increasing background ground-level ozone concentrations. Climate affects ozone production and distribution processes and is therefore expected to affect production and destruction fluxes of ozone and its precursors, and hence their global distributions (Bytnerowicz *et al.*, 2007; Fiore *et al.*, 2002). Over the next century the effects of climate change will become more pronounced and climate driven changes of atmospheric chemistry will become more significant.

The combined effects of greenhouse gases and ozone on vegetation also need to be considered. The effects of CO_2 and O_3 are usually opposing; whilst high concentrations of CO_2 stimulate growth and photosynthesis, O_3 tends to reduce biomass and growth. CO_2 levels are currently on average 375ppb in the U.K., while the average level in pre-industrialised U.K. was 270ppb (IPCC, 2001); this concentration is predicted to rise to 525-750ppb by 2080. The effects of global warming in the U.K. mean that it is expected that temperature will warm on average 2-3.5°C by 2080, winters will be milder, summers hotter and significantly drier, while sea levels will rise on average by 26-86cm above the current sea level (Hulme *et al.*, 2002). All these predictions depend on the emission scenarios used, but indicate that ozone cannot be considered in isolation from other major environmental changes.

1.1.3 Current and future ozone concentrations

Ozone concentrations in the northern hemisphere typically range between 20 and 60 ppb; in addition, on calm, sunny summer days, the concentration may rise and reach levels of up to 250 ppb (Stockwell *et al.*, 1997). In a recent air pollution episode, in the U.K., in August 2003, recorded ozone concentrations were in the range of 90-180ppb over 10 days (Kent, 2003). This episode of ozone coincided with a persistent high pressure system which led to successive days of sunshine and high temperatures (Kent, 2003).

However, there is evidence that peak ozone concentrations have been reduced in the UK by about 30 parts per billion (ppb) over the last decade (NEGTAP, 2001) and ozone precursor emissions were reduced in the EU-15 by 35% between 1990 and 2002 (EEA, 2005). In contrast, there is also evidence to suggest that the UK

and northern hemispheric background levels are increasing by 0.1 ppb y^{-1} (Coyle *et al.*, 2003a).

Ashmore *et al.* (2002) have modelled expected changes in ambient ozone levels up to 2100 in the U.K., assuming a continued gradual increase in northern hemisphere background concentrations; they predict increases of '13%, 29% and 55% from 1996 to 2000 to 2030, 2060 and 2100, respectively, which correspond to a ~4, 8, and 15 ppb increase in mean background concentration'. These increases are expected to be higher, approx. 40ppb by 2030, in windier upland areas, while increases in background concentrations are likely to be at their highest in late winter to early spring (Ashmore *et al.*, 2002). Ozone effects on vegetation are therefore likely to become more significant in the U.K. uplands than other areas of the U.K.

The significance of such changes in background and peak ozone concentrations partly depends on whether there is a threshold for adverse effects on vegetation. Plants have developed mechanisms to deal with ozone and other natural oxidants, and hence some threshold value would be expected. However, the correct value of this threshold for use in risk assessment is less certain. In Europe, the threshold concentration used is 40 ppb, although it is likely that this value can naturally vary between species. The 40ppb value has been used by the United Nations Economic Commission for Europe (UNECE) as a cut off concentration to define a critical level based on an index called the AOT40; a value of AOT40 above this critical level is assumed to cause damage to sensitive plant species (Fuhrer *et al.*, 1997). Currently seasonal values of AOT40 in many parts of Europe exceed the current critical levels for ozone (LRTAP Convention, 2004).

The AOT40 index is based on the accumulated hourly exposure to ozone over 40ppb during daylight hours. Initially the index was derived as a guideline for maximum ozone exposures for crop species; but has since been used to describe dose-response relationships in ozone exposure studies. There is considerable debate over the relative importance of short-term peak concentrations in comparison to long term accumulated concentrations. Recently, there has been further debate as to whether the AOT40 index is truly applicable to studies of semi-natural vegetation and it has been suggested that a lower threshold of 30 ppb should be applied (see reviews by Grunhage & Jager, 2003).

1.2 The Yorkshire Dales National Park (YDNP)

There are many national parks in the UK, which contain protected habitat and seek to provide open spaces for enjoyment of the public. The majority of the UK's national parks are in upland areas e.g. Snowdonia, the Peak District and the Yorkshire Dales. Many of the UK's protected habitats and sites are also in upland areas. Any assessment of the impacts of current and future ozone concentrations on the nationally important conservation of the UK uplands needs to consider these impacts in the context of the wider ecology, history and management of these areas. Since this thesis will focus on ozone in the context of specific habitats of the Yorkshire Dales National Park, the relevant background for this region of the country is considered first.

1.2.1 Introduction

The Yorkshire Dales National Park is an internationally significant upland area for conservation. The YDNP covers 1,773 km^2 of northern England's uplands, situated in the Pennine hills. The peaks rise to 700m above sea level and the

National Park incorporates semi-mountainous habitats as well as lowland habitats in the valley bottoms. The National Park Report (Yorkshire Dales National Park Authority, 2001) states land cover and usage as:- 52% moorland, 40% farmland, 3% woodland, 2.2% rocks, scars and limestone pavement, 0.2% open water, 0.2 % quarries, 0.9% other. Within the park are 101 Sites of Special Scientific Interest (SSSIs), covering some 50,578ha, 5 National Nature Reserve (NNR) covering 1200ha and 5 Special Areas of Conservation (SACs), 1 Special Protected Area (SPA) and 1 RAMSAR site covering 40,066ha (Yorkshire Dales National Park Authority, 2001).

1.2.2 Ecology of the Yorkshire Dales

The Yorkshire Dales overlay a variety of rock substrates but are mainly dominated by the Great Scar Limestone. This was laid down as marine sediment during the carboniferous period. The limestone has a dominant effect on the plant communities present and creates a variety of habitats from calcareous grasslands and meadows to limestone pavements on the exposed areas of limestone karsts.

The northern areas of the YDNP are underlain with sandstones, shales and limestones of the Yoredale rocks; on the east and west sides the limestone is covered with Millstone Grit from glacial drift deposits (Atherden, 1992). Where the Millstone Grit is prominent, the grasslands are acidic and intermixed with moorland vegetation (Atherden, 1992). Acid grasslands cover some 25% of the YDNP (Atherden, 1992). As previously mentioned in the National Park Report (2001), moorland is the most common habitat type making up 52% of the park area. Heaths and moorland are much discussed in literature and are not of interest

for this review, as they are not considered to be of great conservation interest (UK BAP, 2007).

The ecology of the YDNP has been moulded through its long term historical usage for agriculture. This has shaped the landscape and left a legacy of semi-natural habitats from meadows to coppice woodland. The grasslands are largely man-made, the natural habitat being climax woodland from the post glacial period, ash-hazel woodland (Atherden, 1992). Sheep grazing has been common on the Yorkshire Dales since as early as the Iron Age. Industry has also left its impact on the ecology of the YDNP; mainly from quarrying and mining. These have left not only earth works and river diversions, but toxins and pollutants that have had big impacts on the surrounding ecology. The tourist industry is thriving and is an ever growing industry within the YDNP.

Discussion of key habitats in the YDNP is confined to areas of high conservation importance nationally and within this region. These are as follows: hay meadows, limestone pavement and upland woodlands

1.2.3 Hay meadows

One of the most conservationally important habitats in the YDNP is upland hay meadows. This conforms to National Vegetation Classification (NVC) community MG3 *Anthoxanthum odoratum – Geranium sylvaticum* (Rodwell, 1992). Hay meadows make up 30% of all enclosed fields in the YDNP (Atherden, 1992). They are a rare habitat in the U.K. and are mainly confined to upland valleys in northern England (Jefferson, 2005). In the YDNP, the hay meadows tend to lie on the flanks of the rivers, in the low lying valleys; between 200m and 400m

elevation. MG3 meadows are considered to be of high botanical interest and are eligible for SSSI status (Nature Conservancy Council, 1989). Conservation of upland hay meadows is largely through statutory site designation and voluntary agreements set up with landowners. Declines in this community over the past 50 year have been attributed to agricultural intensification, namely the switch to high intensity silage production from low-intensity hay production (UK Biodiversity group, 1998; Blackstock *et al*., 1999; Pacha 2005). Typically the community is now found in small isolated fields or groups of fields surrounded by agriculturally improved meadows and pastures (Jefferson, 2005).

MG3 grassland is characterised by a dense growth of grasses and herbaceous dicotyledons (60-80cm height), with no single grass species consistently dominant, and there is a striking diversity of dicotyledons (Rodwell, 1992). The inability in this type of community for one grass species to dominate may relate directly to the parasitic nature of *Rhianthus minor*, which is frequent within this community, and reduces the competitive ability of grasses (Pywell *et al*. 2004). In addition to this, management practices including, timing of hay cutting, impacts of grazing and fertiliser inputs have been found to alter the diversity and species composition of upland hay meadows (see review by Jefferson, 2005).

1.2.4 Limestone pavements

Limestone pavements are bare expanses of limestone which have been uncovered by the actions of glacial movement and action of ice sheets during the Pleistocene period (1.8 million to 8,000 years ago). The habitat is made of large blocks of limestone called clints and separated by larges fissions or cracks known as grikes. The grikes are formed through water action dissolving the limestone and widening

the cracks. The grikes create woodland like habitat being shaded and humid. This allows refuge for woodland species such as *Mercuralis perrennis, Hyacinthoides non-scripta* and ferns such as *Asplenium trichomanes* and *Phyllitis scolopendrium*. The cracks in the pavement also allow considerable refuge from grazing (Ward & Evans, 1976).

The plant communities on pavements have a tendency to be those with tolerance to drought. There is often little or no soil in these habitats and water will seep through the limestone so there is only limited water available to plants. Regeneration from seed is believed to be the primary source of colonisation in limestone pavements due to continual gap creation caused through drought and frost heaving (Stephenson & Herendeen, 1986; Rusch & Fernandez-Palacios 1995).

In the Yorkshire Dales Biodiversity Action Plan (2004), Limestone Pavement was given a high priority of conservation importance. Limestone pavements are a Priority Habitat type on Annex 1 of the EC Habitats Directive. The importance of limestone pavement in the YDNP arises for the following reasons:

i) The great scar limestone covering North Yorkshire to the Lake District is a unique landscape, being of high altitude, and has not only biological but geological importance (Ward & Evans, 1976);

ii) Cumbria and North Yorkshire share 87% of the UK's Limestone Pavement (Ward & Evans, 1976);

iii) A large proportion of the British populations of *Actaea spicata* (baneberry) occur on the limestone pavements of North Yorkshire (Ward & Evans, 1976).

iv) The total area of limestone in the EU is >3000 ha and the UK holds a significant proportion of this (UK BAP, 2007).

1.2.5 Upland Woodlands

British woodlands have been very much in decline over the last century mainly due to changes in land use and the replanting of native woodland with conifer plantations. Upland broadleaved woodlands are a scarce habitat in the U.K., with upland coniferous woodlands being more frequent. Upland semi-natural woods have declined by about 30-40% in area over the last 50-60 years as a result of replanting, mainly with introduced conifers, clearance for quarries or other developments in some areas, and from conversion to rough grazing (UK BAP, 2007). The habitats of importance in the YDNP are mixed ash woodlands (W8 *Fraxinus excelsior - Acer campestre - Mercurialis perennis* woodland and W9 *Fraxinus excelsior - Sorbus aucuparia - Mercurialis perennis* woodland), together with W13 *Taxus baccata* woodland for the yew groves on the Carboniferous and Magnesian limestones. Less frequent sub-communities, that may occur in mosaic with the above, are relatively dry alder-ash stands (W7c and the more southerly and eastern sub-communities of W8 (a-c)) and upland oak woodlands (no NVC designation). Both habitats are among of the richest habitats in the UK for plant diversity (UK BAP, 2007).

A recent study of changes in British woodlands over the last 30 years by Kirby *et al.* (2005) has suggested that the major drivers of change have been those deriving from increasing pH from eutrophication via nitrogen deposition and from lack of management resulting in increasing shade within some woodland. Kirby *et al.* (2005) state that increasing basal areas in woodlands are leading to a more shaded

environment for ground flora. The consequences of this over the last 30 years is a shift towards more shaded assemblages of woodland plants. Overall the study reported a 12% decrease in species richness at the study sites over a 30 year period (Kirby *et al.,* 2005).

Peterkin (1996), in a review of woodland conservation, outlines three process to enhance and maintain biodiversity; creating a framework for British woodland conservation objectives:

i) protection of areas of ancient semi-natural woodland and associated management practices;

ii) conservation integration into modern commercial forestry; and

iii) creation of new woodland habitat.

1.3 Ozone and semi-natural vegetation

There is limited information on the effects of ozone exposure on semi-natural vegetation and even less information that relates to studies of the communities of interest in the YDNP. Three major limitations of the available information can be identified. Firstly, many studies have been conducted in indoor fumigation systems, or in outdoor open top chambers and solar domes (e.g. Peijel and Dannielsson, 1997; Hayes *et al.*, 2006a), which do not replicate exposure conditions in the field. Secondly, many studies are for a limited time period; in contrast to arable crop species, for which measuring growth parameters and yield over a season is a suitable method for assessing sensitivity to ozone, the effects of ozone on semi-natural vegetation may be cumulative over long periods. Finally, most studies are of individual species or artificial species mixtures, which do not represent the real competitive environment.

These factors create major difficulties in assessing impacts of ozone exposure on the long-term survival and sustainability of wild populations of plants, which also affect the experiments and field studies described in this thesis. It is important for experimental studies to target species in ways that relate to their natural habitat. Therefore, rather than provide a detailed review of all ozone effects on semi-natural vegetation (*for ozone effects on wild species e.g.* Davidson and Barnes, 1998, *for grasslands e.g.*, Bassin *et al.*, 2006; *for trees e.g.*, Matyssek and Sandermann, 2003;), this section will consider some of the major impacts and processes in terms of their relevance to the specific communities of the YDNP.

1.3.1 Ozone induced foliar injury

A typical response of vegetation to exposure to ozone is visible foliar injury. Symptomatic ozone induced foliar injury typically takes the form of stipples, flecks or bronzing (Skelly *et al.*, 1999). Gravano *et al.* (2003) reported damage in 'stippled' *Ailanthus altissima* leaves to be a result of loss of chlorophyll in the palaside mesophyll cells and damage to organelles. In *Apocynum androsaemifolium* (spreading dogbane) fine brown to black adaxial stippling occurs over large areas of the leaf following exposure to elevated ambient levels of ozone; this leads to chlorosis and premature senescence of older affected leaves (Bergweiler and Manning., 1999). Plants also exhibit general visible signs of foliar stress when exposed to ozone such as: - chlorosis; increased levels of anthocyanins causing redness (Foot *et al.*, 1997; Bergmann *et al.*, 1995); and premature senescence (Bergmann *et al.*, 1995; Franzaring *et al.*, 2000).

Foliar injury is not always associated with reductions in biomass or growth, and species exhibiting reductions in biomass do not always exhibit foliar injury (Bassin *et al.*, 2006; Davison and Barnes, 1998). It was concluded by Davison and Barnes (1998) that visible symptoms are best regarded as evidence of a biochemical response to ozone; they are not evidence of an ecological impact.

1.3.2 Individual species growth responses

A number of studies have reported the effects of ozone on the growth of individual plants of species of semi-natural vegetation. A recent meta-analysis of these data identified 83 species with relevant data, and sought to derive an index of relative sensitivity based on a dose-response relationship (Hayes *et al*; 2006b); further to this, the study highlighted traits of species, aiming to identify

physiological and ecological characteristics that would identify them as likely to be sensitive to ozone. The main findings were as follows:

i) Species from the *Fabaceae* were more likely to be sensitive to ozone than those species from *Asteraceae*, *Carophyllaceae*, and *Poaceae*;

ii) Relative sensitivity to ozone compared with Ellenberg ecological habitat scores suggest that: light-loving plants tended to be more sensitive to ozone than shade species; high drought tolerant species tended to be more sensitive than those found in moister soils; and species associated with saline conditions were more sensitive than those from less saline habitats;

iii) Consistent with other studies (Gimeno *et al.*, 2004; Pleijel and Danielsson, 1997; Warwick and Taylor, 1995) there was no correlation between relative sensitivity and C-S-R strategy (Grime, 2002);

iv) There was no correlation between relative sensitivity to ozone and mature leaf Phosphate concentrations, leaf longevity, flowering season, stomatal density for upper and lower surfaces, and maximum altitude.

Hayes *et al.* (2006b) found no correlation between maximum altitude at which a species may be found and relative sensitivity to ozone; this suggests that species from upland habitats are unlikely to be affected by ozone merely due to adaptations to environmental conditions at higher altitude.

Species with a therophyte (annual) life form and species which are typical of light environments are also predicted to be more sensitive to elevated ozone (Hayes *et al.*, 2006b). Furthermore, evidence suggests genotypes and species with a high growth rate are more sensitive to ozone than slow-growing ones, due to a higher

gas exchange rate, accompanied by a higher uptake of ozone or a lower ability to re-allocate resources when exposed to stress (Reiling and Davison 1992a; Danielsson and Pleijel 1999; Manninen *et al.* 1999; Bortier *et al.* 2000).

The majority of species listed in the NVC categories representative of the upland limestone pavements and hay meadows are generally long-lived perennial species whose survival strategy relies on vegetative growth and asexual reproduction in addition to seed production; these are generally not representative of therophyte strategists. However, the species common of the early succession habitats in woodlands are typical therophytes.

1.3.3 Reproduction

There is evidence of effects of ozone on several reproductive parameters including:- the number of inflorescences (e.g. Chappelka (2002) found elevated ozone resulted in initial increases in the flowering of *Rubus cuneifolis*); on the number of fruit and/or seed formed (e.g. Bergmann *et al.*, 1995, 1996; Bender *et al.*, 2006; Pearson *et al.*, 1996); and in the germination ability of seed from ozone exposed parent plants (Bender *et al.*, 2006). There is also some evidence that the ozone sensitivity of semi-natural vegetation tends to increase during the reproductive phase of plant growth; Bassin *et al.* (2004) reported greater sensitivity to ozone exposure in *Centaurea jacea* during the reproductive stage.

The study by Bender *et al.* (2006), on the effects of ozone exposure on 17 species of European wild plants, demonstrated the potential for many species of semi-natural vegetation to have reduced seed output after ozone exposure. Furthermore, many of the species studied showed reduced germination ability of seeds from

ozone exposed parent plants (Bender *et al.*, 2006). Reduction in seed production can also vary within a species, the study by Pearson *et al.* (1996) looking at three *P.major* populations showed differing effects of ozone on the output of seed between populations. For a comprehensive review of effects of ozone on reproductive development of plants see Black *et al.* (2000).

1.3.4 Carry-over effects

Ozone exposure may have longer-term effects on growth and fecundity for longer lived species. The effects of ozone stress, and of detoxification, compensation and repair induced by ozone exposure, may have negative effects for the following year's growth. Reductions in carbon partitioning to the roots and the effect of ozone on phloem loading, which are both well-established effects of ozone, (see review by Fuhrer and Booker, 2003) may result in long-term changes below ground. This effect may be more pronounced in species which rely on carbon sinks in the roots or bulbs, for subsequent regeneration; in particular exposure may have a greater effect on spring flowering bulbs, which regenerate during spring, when future ozone exposure levels are predicted to be highest.

Franzaring *et al.* (1999) suggested that recurring high concentrations of ozone in spring might pose a threat to the young leaves of perennial plants as their vegetative parts may have 'memorised' the previous season's ozone stress. However, the sensitivity of perennial plants seems to be higher in the first year of exposure and a decrease in ozone response in subsequent years has been consistently reported in multi-year exposure experiments (e.g. Tonneijck *et al.*, 2004; Barbo *et al.*, 1998; Bungener *et al.*, 1999).

Hayes *et al.* (2006a) exposed thirty-three species, which were collected from wild populations from the Snowdonia National Park, over a 10-week exposure period in solardomes and an over-wintering period exposed to ambient air; these species were representative of NVC CG10-12 (*Festuca ovina – Agrostis capillaris – Thymus praecox* grassland*; Festuca ovina – Alchemilla alpine – Silene acaulis* dwarf herb community (Rodwell, 1992)). They reported significant carry-over effects on regrowth in the following season for three perennial species of U.K. upland vegetation: *Gallium saxatile, Nardus stricta, and Saxifraga stellaris*. In addition, within the growing season, they found a significant 33% reduction in above ground biomass of *Juncus effusus* and 97% reduction in *Saxifraga stellaris* in ozone exposed plants (Hayes *et al.*, 2006a).

In contrast to these results, Tonneijck *et al.* (2004) found no evidence of reduced biomass following over-wintering for *Plantago lanceolata, Holcus lanatus, Lychnis flos-cuculi, Agrostis capillaris* and *Molina caerulea* over a three year study with higher exposure levels. Franzaring *et al.* (1999) reported a stimulatory effect of ozone exposure on subsequent year's growth of two wet meadow species (*Centaurea nigra* and *Molina caerulea*) and decreases in growth rate of one species (*Cirsium dissectum)*. The study also looked at the seed germination viability of ozone exposed plants and found no correlation between germination potential and parent plants' ozone exposure (Franzaring *et al.*, 1999). Carry-over effects appear to be variable between species and studies.

1.3.5 Community Reponses

It has been predicted that species sensitive to ozone will be eliminated from communities which are exposed to high levels of ozone (Duchelle *et al.*, 1983;

Armentano & Bennet, 1992; Nebel & Fuhrer, 1994). However, only one study has demonstrated such an effect in real communities; in a calcareous grassland community, Thwaites *et al.* (2006) reported the complete loss of one species (*Campanula rotundifolia*) from ozone-exposed mesocosms.

However, the effect of ozone on community composition and diversity is not properly understood. Species which have shown effects of ozone when grown individually have been found to react differently to ozone exposure when in a community setting (see e.g. Fuhrer *et al.*, 2003) and some 'sensitive' species have shown no response to ozone exposure when grown in a community (Evans and Ashmore, 1992; Ashmore and Ainsworth, 1995). Most studies focus on reductions in biomass, and the effect of ozone exposure on biomass in grassland community mesocosm experiments tends to favour grasses over forb species (e.g. Fuhrer *et al.*, 1993). The text below considers the evidence of effects of ozone on species composition for two major communities of the YDNP:- grasslands and woodlands.

Grasslands

The research on ozone effects on grassland communities is extensive. However, little relevant work has been conducted to assist in predicting effects on U.K. upland grassland. This section focuses on ozone exposure of grassland communities, and on effects on upland or calcareous grasslands and hay meadows, for comparison to YDNP communities. For a comprehensive review of ozone effects on grasslands see Bassin *et al.* (2006).

There is evidence that meadow and grassland species may be sensitive to ozone; Warwick and Taylor (1995) showed that several of the characteristic species of lowland calcareous grassland, including *Anthyllis vulneraria, Festuca ovina* and *Lotus corniculatus*, which are also common in upland hay meadows, were affected by levels of ozone found in the southern and eastern areas of the UK. In addition Hayes *et al.* (2006a) reported for UK upland grassland species: reductions in above-ground biomass in 15% of species tested, visible injury in 24% of species, and reductions in over-wintering ability

In community mixture or mesocosm experiments, a frequently recorded effect of ozone exposure in grassland communities is a reduction in forb species and increase in grasses (Bassin *et al.*, 2006). This has been reported in lowland hay meadows in Finland (Ramo *et al.*, 2006) and in various semi-natural grassland studies (Evans & Ashmore, 1992; Ashmore & Ainsworth, 1995; Samuelsson *et al.*, 2006).

Ramo *et al.* (2006) exposed meadow mesocosms to elevated levels of ozone over three seasons; AOT40 exposure in the different seasons ranged from 85 to 674 ppb.h in the ambient O_3 treatments and from 3132 to 10331 ppb.h in the treatments receiving supplemental O_3. They reported a 40% reduction in above-ground biomass mainly due to reductions in the major forb species *Campanula rotundifolia, Fragaria vesca, Trifolium medium* and *Viccia cracca*. In contrast to these reductions in above-ground biomass, they found no reductions in total community below-ground biomass (Ramo *et al.*, 2006).

Evans and Ashmore (1992), investigating ozone effects on the structure of a semi-natural grassland community, suggested that drivers of change within the grassland sward during ozone treatment were actually fluctuations in the cover of dominant species affected by ozone exposure. They suggested that ozone is not the the major driver of community change but that the composition of the community is dependent on structure, climate and population dynamics, all of which may modify the impacts of ozone (Evans & Ashmore, 1992).

As well as changes to community composition, effects of ozone exposure on the soil N pool have been reported (Ramo *et al.*, 2006). This is likely to further impact community composition, diversity and species number indirectly, as the species in the community react to changes in the nutrient pool. Ozone effects on community composition may well be altered by the effects of grazing or hay cutting in managed grasslands. Fuhrer *et al.* (1993) reported reductions in yield of clover, in response to ozone exposure in grassland community, over a two season mesocosm experiment in which the forage was removed systematically 4-5 times per year. It was suggested that ozone will have a greater effect on community composition in managed grassland systems where the effects interact with cutting or grazing.

Additionally, effects of ozone exposure on grasslands may be modified by environmental conditions. Franzaring *et al.* (2000) found, in a study of two fen-meadow community species, that ozone pollution had a larger impact when the species were under water stress. This was in contrast to the predicted outcome; Franzaring *et al.* (2000) had predicted that wetland species would be more susceptible to ozone when the water supply was adequate, thus allowing greater ozone flux through the open stomata.

Woodlands

The impact of ozone on forest tree species is an extensively studied area and is particularly well documented in North America (Karnosky *et al.*, 2007; Bytnerowicz *et al.*, 2007; McLaughlin and Percy, 1999) and in Europe (Bytnerowicz *et al.*, 2007; Matyseek and Innes, 1999). Much of the research is concerned with effects on coniferous species and there are very few studies focussing on the effects of ozone on European broadleaved woodland and even fewer in Britain. Of the studies in the U.K., most are focussed on lowland woodland in Southern England (e.g. Stribley & Ashmore, 2002). There are no studies looking at the effects of ozone exposure on upland broadleaved woodland and none looking at the effects of woodland management practices and consequent tree regeneration and how this may interact with ozone exposure. In addition to this, most effects are reported from experiments with young trees and these may not be representative of effects on mature woodland trees (Matyseek and Innes, 1999).

Furthermore, there are is almost no information on ozone effects on woodland ground flora. The only major study is that of Barbo *et al.* (1998), who investigated the effects of ozone exposure on an early successional plant community, arising from the site of a cleared 50-year-old North American coniferous forest. The experiment, using open-top chambers (OTC) over the existing ground flora of the forest, was run for 2 years with the highest ozone treatment reaching a maximum AOT40 of 29 ppm.h (Barbo *et al.*, 1998). The experiment is relevant to ozone exposure effects on upland deciduous woodland ground flora, as, although the

woodland was a coniferous one, the dynamics of the community would be similar to that of coppice community post-coppice in the regeneration phase.

Barbo *et al.* (1998) found that cover of *Rubus cuneifolius* was most affected by ozone; in the summer months of the study, cover of *R. cuneifolius* was 2.8 times and 2.4 times higher than the ambient air plots, in concurrent growing seasons in the highest of the ozone treatments. They found no effect of exposure on species richness, but reductions in species diversity in the ozone treatments between years, equating to 20% in the highest treatment; in addition to this, evenness was lower in the ozone treatments (Barbo *et al.*, 1998), suggesting that ozone treatment favoured domination by vigorous herbs.

In the U.K., the effects of bramble *(R. fructicosus (agg.))* dominance on the diversity of woodland ground flora can be severe. The coppice regeneration phase is associated with the dominance of *R. fructicosus (agg.)*; for example, at Bovingdon Hall woods, Essex, four-fold increases in this species in the forth year post-coppice caused significant reductions in species diversity (Mason & Long, 1987). Rackham (1980) suggests that dominance of brambles may be related to the pH of the soil; a pH of 3.5-4.5 (typical of coniferous plantations) stimulates growth of brambles. Therefore the dominance exerted over the ozone exposed communities in the study by Barbo *et al*, (1998) could be a result of suitable condition of growth for *R. cuneifolius* and the lack of competition due to ozone effects on other woodland species. Barbo *et al.* (1998) also reported significant foliar injury on *R.cuneifolius* in the ozone exposed plots; however this did not result in reductions in cover for this species.

It is not clear what the impact of ozone on British woodland ground flora may be, as there is a serious lack of research in this area. However, Barbo *et al.* (1998) concluded that ozone exposure of the successional community that they studied led to changes in diversity and abundance and it would not be daring to suggest that similar responses could be seen in British woodlands. The sensitivity to ozone of British mature woodland broadleaved trees is still unknown, but changes in tree cover, diversity and canopy structure in response to ozone exposure are unlikely to be helpful to conservation of the ground flora.

1.4 Ozone sensitivity, resistance and genetics

There is significant evidence to suggest that different genotypes, ecotypes and populations of individual species may vary in their sensitivity to ozone (Lyons *et al.*, 1997; Bassin *et al.*, 2004; Bungener *et al.*, 2003; Nebel and Fuhrer, 1994). A recent review concluded that:- 'previous exposure to ozone stress may change the genotypic composition of a population through selection of ozone resistant genotypes' (Bassin *et al.*, 2006).

Pearson *et al.* (1996) exposed three U.K. populations of *P. major* (one from the southern lowlands, one from the northern uplands and a third from lowland Scotland) to ozone; they found differing responses between populations dependent upon which measure of performance was examined. It therefore seems that ozone resistance may be present within specific populations, but it is more complex to identify than through growth parameters alone. Pearson *et al.* (1996) found changes due to ozone exposure in *P. major* for: leaf senescence; leaf size; root, shoot and seed biomass; and changes in stomatal conductance; these varied between populations in sensitivity to ozone exposure.

Lyons *et al.* (1997) assessed the effects of ozone exposure on 22 populations of *Plantago major* and found a positive relationship between relative ozone resistance and levels of ozone recorded at the site of seed collection. Lyons *et al.* (1997) suggest that current ambient levels of ozone in the UK are high enough to drive selection of ozone resistant genotypes. There is a question as to whether ozone resistance derives from an adaptation to ozone or an adaptation to other environmental variables which offer resistance. However, Lyons *et al.* (1997)

found no correlation between ozone resistance and other environmental variables, supporting the theory that ambient ozone levels are driving selection within *P.major*.

A similar response to background ozone levels was seen in the difference in response to ozone exposure between populations of *Elymus glaucus L.* from contrasting environments (high and low ozone); the population from a low ozone environment was reported to have greater reductions in the arbuscular mycorrhizal colonization due to ozone than the high ozone population (Yoshida *et al.*, 2001). Analysis of soil characteristics from this low-ozone population of plants revealed also a significant reduction in active soil bacterial biomass and an increase in total fungi per gram dry weight soil (Yoshida *et al.*, 2001).

Pearson *et al* (1996), looking at the effects of ozone exposure on three populations of *P. major* from the U.K., found different responses below ground to those above-ground, specifically in the lowland population. In addition to providing evidence for historical ozone climate eliciting different responses of plant populations to ozone, this suggests a possible role for ozone in altering soil processes and changing the microbial community, which is also related to host plant genetics

Not all studies or species show variation in their sensitivity to ozone between populations. For example, Danielsson *et al.* (1999) found no difference in ozone sensitivity between 9 genotypes of *Phleum pratense* and three genotypes of *P. alpinum*, although both species did exhibit a negative effect of elevated ozone on above-ground biomass. Bassin *et al.* (2004) also found no correlation between

native ozone climate and sensitivity to ozone of populations of *C.jacea*. One explanation is that some species may contain limited genetic plasticity and thus be unable to develop resistance. Species such as *P.major*, which has been consistently studied, are typically plastic in their response to environmental variables and the responses of such species may not be a reflection of all plant species responses to ozone.

In summary, there is evidence that variation in resistance or sensitivity to ozone may exist between and within populations of some species. It should be of primary importance when considering ozone impacts in a particular region, such as the YDNP, to ensure that stock for experiments comes from wild populations in that region. It also follows that it is important to ensure when screening species which are present in multiple habitats that as many of the ecotypes as possible are present within a study. It is possible that a species that is considered insensitive in one habitat may be sensitive in another.

1.5 Likely implications for ozone impacts in the YDNP

Upland hay meadows are managed by grazing of cattle and sheep, and by cutting for hay and silage. In addition to gaps created through natural processes, grazing and cutting both create gaps in vegetation and this allows for regeneration of grassland via vegetative expansion of perennials and germination of seeds from the seed bank. In Northern climates, natural regeneration is usually seasonal and occurs in the spring (Grime, 2002). It is possible that additional gaps for regeneration could be created in such habitats through the effects of ozone on over-winter survival of perennial species such as those reported by Hayes *et al.* (2006a).

Typical limestone grassland species rarely possess persistent seed banks; they are small and short-lived (Akinola *et al.*, 1997), as are many grassland seed-banks (Chippindale & Milton, 1934; Major and Pyott, 1966; Hayashi and Numata, 1971; Donelan & Thompson 1980; Graham & Hutchings 1988; Thompson *et al.* 1998). They are typically of a transient nature that is characteristic of permanently closed vegetation (Thompson & Grime, 1979). In contrast, woodland seed-banks comprise the transient seed banks of the late successional, shade tolerant flora and the persistent seed banks of intermittent marginal flora of light demanding species (Brown & Warr, 1992). Hay meadow species in the YDNP are confined to small habitat patches (Jefferson, 2005); it is likely that little or no migration between populations will occur in the less common and more specialised meadow species. Without a persistent seed-bank, low reproductive success in upland hay meadow species, due to ozone exposure, could quickly lead to invasion of ruderals and species common of disturbed habitat sites, e.g. *Cirsium, Epilobium.*

Recovery of the community will be difficult once damage is caused, due to habitat fragmentation, low migration rates and lack of a persistent seed bank. The potential risk to such communities from ozone could lead to: decrease in population size of meadow species, decreases in biodiversity and degradation of habitat.

The same scenario exists for woodland species; low fecundity could lead to changes in biodiversity and dominance of a community. Reproductive effects on wild plants are wide ranging and due to the direct effects on reproduction are likely to drive selection quickly in areas with high exposure. This will leave communities with low exposure, perhaps due to sheltering effects of canopy cover or in communities in sheltered valley bottoms, more susceptible to seasonal peak episodes.

The likelihood is, due to the fragmentation of habitat in the YDNP specifically that of high quality hay meadow habitat and upland broadleaved woodland, that the most vulnerable species ecologically will be the long-lived perennials that do not form a persistent seed-bank and are comprised of small localised populations. This is because small plant populations are more prone to extinction, due to the loss of genetic variation through random genetic drift, increased selfing, and mating among related individuals.

1.7 Aims and Objectives

Davison and Barnes (1998), following a review of ozone effects on wild species, suggested that there is a pressing need to evaluate the risk to natural vegetation posed by ozone. There is little research looking at the effects of ozone on semi-natural vegetation compared to that recorded for crops and forests. Further to this, Davison and Barnes (1998), state that, because of the immense number of wild species and the variety of conditions under which they grow, it is necessary to target investigations on the most sensitive taxa, ecosystems and processes. With ozone levels at their highest in upland areas, species in these areas (which are of high conservation importance) may be among the most vulnerable. While, there have been many studies looking at ozone effects on lowland grassland communities, and there has been much research on forest trees, there is very little on upland communities of conservation importance such hay meadows, limestone pavement and woodland ground flora.

Therefore, the main aim of the research described in this thesis was:- to contribute to the assessment of the impacts of ozone on upland habitats, and in particular on characteristic communities and species of conservational importance in the YDNP,

The specific objectives of the research were as follows:-

- *To compare the sensitivity to ozone of plants from different communities within the YDNP.* While published studies have compared populations from different locations, none has specifically set out to test if species and

populations from different habitats have significant differences in ozone sensitivity.

- *To assess the effects of ozone on woodland bulb species.* There are no published studies of the effects of ozone on U.K. spring flowering bulb species.

- *To investigate how woodland and grassland canopies modify ozone exposure and risk of damage to vegetation*, through measuring current levels of ozone at sites in the YDNP. There is little information on ozone exposures within woodlands and therefore on the exposure of woodland ground flora.
- *To evaluate the effects of ozone on the species composition of emerging woodland ground flora communities.* Since the effects of ozone can be altered by the effects of land management and environmental conditions, and since in the U.K. most broadleaved woodland are under some form of management, generally rotational coppicing, these studies included interactive effects with shade and a comparison of communities from different stages of the coppice cycle.

Given the importance of placing the effects of ozone described in Section 1.3 in the specific ecological context of the YDNP, as described in Section 1.2, the research aimed consistently for an approach of maximum ecological relevance. For example, it is clear that below-ground responses to ozone as well as those above-ground are important to the success of individuals and populations. Ozone alters resource allocation patterns; the direction of the effect is highly dependent on species, population origin and on environmental conditions. The effects of

ozone within communities are not clearly understood and the outcomes of competitive interactions may be modified by ozone exposure. In addition to this various environmental factors such as light and water availability may alter the effects of ozone exposure on semi-natural vegetation. Combined, these factors may alter the ability of species or populations to survive or successfully compete in specialised niches, such as woodland and limestone pavement communities.

1.8 Structure of Thesis

Chapter 2 seeks to compare the ozone sensitivity of species taken from communities in areas of high conservation importance in upland semi-natural vegetation specific to the YDNP, with a particular focus on population differences. Fourteen species of upland vegetation, selected from six SSSIs, representing upland grasslands, hay meadows and woodland, were exposed to ozone in a short-term growth experiment in an indoor fumigation system with young plants. Three species were collected from multiple habitats and comparisons were made between populations.

Chapter 3 is an investigation into the effect of ozone exposure on spring flowering bulbs species that are common within limestone pavements and woodlands. Two flowering bulb species were collected from an upland woodland in the YDNP, namely *Hyacinthoides non-scripta* and *Allium ursinium*; and *Narcissus pseudonarcissus* was obtained from a commercial source. These were then exposed to ozone from emergence from the buried bulbs for 24 weeks, and growth parameters were measured and assessed.

The focus of *Chapter 4* is a field investigation of ozone concentrations and stomatal conductance in woodland herbs and in hay meadow species in woodland and grassland canopies within the YDNP. Wild species differ greatly in stomatal conductance and leaf anatomy (Davison and Barnes, 1999), while the depth of canopy and leaf area index formed by other grassland and woodland species may alter the flux of ozone to leaves within the canopy through changes in both ozone concentrations and stomatal conductance (Jaggi *et al.*, 2006; Karlsson *et al.*, 2006).

Chapter 5 reports the results of three mesocosm experiments to examine the effects of ozone exposure on a woodland ground flora community and its interaction with shade. This study assessing the effect of ozone exposure on woodland communities, was undertaken to pursue the sensitivity of individual woodland species to ozone that was indicated by the results in *Chapter 2*. The shade treatment in this study aimed to simulate the effects of a growing tree canopy in spring. In contrast to published mesocosm studies, which have either introduced plants artificially or used an established community, species were allowed to emerge from soil collected from a wood within the YDNP, to simulate effects of ozone on the development of a woodland community. Soil was collected from different areas of the wood, representing different stages of the coppice cycle, to assess if this modified responses to ozone.

Finally, *Chapter 6* discusses the findings of this research, aiming to place them into a wider ecological context.

2. Chapter 2: Sensitivity screening to ozone exposure of U.K. upland vegetation

2.1. Introduction

As described in Chapter 1, the Yorkshire Dales National Park (YDNP) is an area of high conservation importance in the U.K, which contains many habitats that are scarce or rare within the U.K. (Yorkshire Dales National Park Authority, 2007). Ozone exposure in upland and remote areas of the UK is expected to increase over future decades, due to increases in tropospheric background levels (Coyle *et al.*, 2003). This makes habitats of high conservation value within such areas particularly at risk.

Spatial differences in response to ozone within an upland region of conservation importance depend on a number of factors. One of the most important of these is clearly the sensitivity of the individual species found in each community. Large differences between the sensitivity of wild species have been identified, although there is little evidence that ozone sensitivity of a species is strongly related to specific species attributes, such as growth strategy (e.g. C-S-R types) or Ellenberg habitat indicators (Bassin *et al*, 2006; Hayes *et al.*, 2006b).

There is also evidence in the literature of differences in ozone sensitivity between populations of the same species collected from different locations (e.g. Lyons *et al.*, 1997; Pleijel & Danielsson, 1997). These differences have been primarily interpreted in terms of variation in the ozone exposure leading to evolution of ozone tolerance in populations experiencing greater exposure. However, none of

these studies have specifically tested whether these differences are systematically related to the habitat from which species have been collected, and there is a possibility that adaptations to particular habitat conditions may lead to differences in sensitivity to ozone exposure.

Most studies of plant responses to ozone have focused on effects above ground, but the ecological fitness of populations may depend on a range of other response variables which are less commonly assessed (Davison & Barnes, 1998). Effects on root growth may be of considerable ecological significance in the characteristically nutrient limited communities of upland Britain. Furthermore, in the freely drained limestone soils that are characteristic of many areas of the YDNP, intermittent water stress during the summer months may be a significant ecological factor

Given this background, the aims of this experiment were to:-

- identify sensitive species of upland communities of this area;
- compare the ozone sensitivity of species and populations of different habitats;
- assess if the effects of ozone on species of upland habitats were greater on below-ground than above-ground growth.

2.2 Methods

2.2.1 Plant Selection

Plant species for this study were selected using the following three criteria:-

i) Species must be characteristic of the upland environment in the Yorkshire Dales and characteristic of relevant National Vegetation Classification (NVC) classifications (Rodwell, 1992). These are: calcicolous grassland (specifically NVC communities: CG9, CG10 and CG2); mesotrophic grassland (specifically upland hay meadow NVC community MG3); upland broadleaved woodland areas and limestone pavement. All of these are Annexe 1 habitat types within the EU Habitats Directive.

ii) The species must include examples from a variety of genera, but should exclude grasses as these are a much studied group of species (Hayes *et al.*, 2006b; Bassin *et al.*, 2006)

iii) They must include, if possible, rare or scarce species.

The final selection was based on these criteria but also was greatly dependent upon the presence of the species at sites selected for study.

2.2.2 Site Selection

Sites were chosen to represent habitats typical of the upland areas of the Yorkshire Dales National Park (YDNP). It was also important to include sites that

were botanically diverse and representative of endangered and protected UK habitats. All sites that were selected had SSSI status to fit these criteria.

A total of six sites were selected, which are described in greater detail below. Upland hay meadow and grasslands are present in good condition at Yockenthwaite Meadows. Grass Wood, Conistone Old Pasture (COP) and Bastow Wood lie alongside each other and are three very different habitats which merge and blend into each other. The final two sites are part of the Craven Limestone complex (Special Area of Conservation) and Ingleborough National Nature Reserve; Colt Park Wood and Scar Close are areas of limestone pavement.

2.2.2.1 Yockenthwaite Meadows (SD 911786)

Two grassland NVC communities dominate this site (Natural England, 2007):

i) **MG3**: *Anthoxanthum odoratum - Geranium sylvaticum* grassland.

ii) **MG5**: *Cynosurus cristatus - Centaurea nigra* grassland.

These meadows occupy an area of 11.2ha, and are located on steep south facing slopes in Langstrothdale; they lie adjacent to the river and up to an altitude of approx. 300m. Yockenthwaite Meadows are owned by the National Trust and managed as traditional hay meadow; they are also grazed by sheep. The meadows are particularly species rich and diverse with typical upland hay meadow species.

2.2.2.2. Conistone Old Pastures (SD 990670)

This site is dominated by two NVC communities (Natural England, 2007):

i) **CG9** *Seslaria albicans – Galium sterni* grassland.

ii) **U4** *Festuca ovina - Agrostis capillaris - Galium saxatile* grassland.

Conistone Old Pastures cover a large area from Bastow Wood (see below) to the small village of Conistone on the eastern flanks of the Wharfe valley. They are diverse in habitat type, ranging from managed pasture on the lower reaches to

grassland and limestone pavement on the upper reaches, with scattered areas of moorland. The pastures are scattered with large deposits of scree which provide habitat diversity allowing species such as *Geranium robertainium* and *Minuarta verna* to join the grassland communities here. The site is primarily important for its geological features namely the limestone scars that cap the valley and the dry waterfall, Dib Scar, which divides the pastures from the neighboring Bastow Wood.

2.2.2.3 Bastow Wood (SD 990657)

This site is dominated by three NVC communities (Natural England, 2007):

i) **W13** Yew Woodland alongside Mixed broadleaves;

ii) **CG9** *Seslaria albicans – Galium sterni* grassland;

iii) **U4** *Festuca ovina - Agrostis capillaris - Galium saxatile* grassland.

Bastow Wood comprises two distinct habitat types the mixed broadleaved upland woodland, which is also typical within Grass Wood (see below), and CG9 and U4 grassland communities which are typical of Conistone Old Pastures (see above). The most diverse areas lie on top of the plateau, where the woodland is more like wood pasture with much open space. The sward in generally low to the ground, being mainly controlled by grazing rabbits although sheep graze at the site from time to time. Species such as *Primula farinosa* and *Primula veris* are common on these upper reaches of the wood. The habitat diversity is good, ranging from bare limestone to wood-pasture

2.2.2.4 Grass Wood (SD 985655)

This woodland site is dominated by two NVC communities (Natural England, 2007):

i) Mixed Upland Broadleaved Woodland

ii) **W13** Yew Woodland

Grass Wood is owned by the Yorkshire Wildlife Trust. It is mixed oak-ash woodland on limestone. The gradient is steep and it runs down to the River Wharfe. On the top of the plateau is Bastow Wood, which neighbours Grass Wood. Grass Wood is notably rich in species, with many common woodland species. Its particular geography allows higher species diversity due to the habitat mosaic, as, for example, limestone pavement species such as *Geranium robertainium* and *Lotus corniculatus* become incorporated into the woodland flora. The woodland also has rich carpets of spring epiphemerals such as *Mercuralis perrenis*, *Hyacinthoides non-scripta*, *Allium ursinium* and *Primula vulgaris*. *Paris quadrifolia*, is also present in the woodland. There is a small area of the woodland which is being traditionally coppiced and much of the planted conifers on the site are presently being removed to increase the biodiversity of the site. Much of the site is made up of mature stands with little light breaking through the canopy in the summer, except around the woodland edge and in the rides. The rides are thus important hot-spots for diversity with most species being present in cleared areas and the rides.

2.2.2.5 Craven Limestone Complex (SAC) and Ingleborough National Nature Reserve

Ingleborough is a large area of the YDNP comprising areas of limestone pavement and escarpments. Scar Close and Colt Park Wood are two sites within the NNR at Ingleborough which also is a component of the Craven Limestone Complex Special Area of Conservation (SAC).

2.2.2.6 Colt Park Wood

There are two dominant NVC communities: (Natural England, 2007):

i) **MG6** *Lolium perenne - Cynosurus cristatus* grassland;

ii) Upland Limestone Pavement Woodland

English Nature's office at Ribblehead is a barn situated adjacent to Colt Park Wood SSSI. The field surrounding the office belonging to English Nature was used as the study site. This site is a mix of limestone pavement and meadow, grazed and cut for hay. The site is not particularly rich in species, but does provide some of the properties of the nearby SSSI which is treacherous and unsafe to enter.

2.2.2.7 Scar Close (SD 760740)

This site features diverse habitats with a wide range of species present. These include upland species common of rocky habitats combined with species more common within woodland habitats. At Scar Close, the limestone is broken up by islands of acidic peat where moorland species also add to the floristic diversity. The depth of the grikes varies considerably across the site, from some very deep grikes unable to support any plant life, to shallower grikes, which provide suitable habitat for shade-tolerant woodland species. The peat islands support large areas of *Calluna vulgaris*. The site is becoming woodier, with *Sorbus aucuparia* and *Corylus avellana* becoming more common, due to cessation of grazing on the site.

2.2.2.8 Malham (SD 920676)

The Malham area is a large region covering areas of ancient limestone cliffs, lowland wet woodlands, waterfalls and a large tarn with adjoining moor land. The seeds used were collected by a colleague from the woodland area around Janet's Foss and this was not intentionally a study site.

2.2.3 Seed Collection

Between the months of May to September 2003, seeds were collected from wild populations at the six sites within the Yorkshire Dales National Park. A standard collection protocol was used; seeds were collected at 5 minute intervals on a planned walk through a site. However, some species, e.g. *Anthyllis vulneria,* were only collected from specific areas at one site due to the scarcity of the species. Seeds collected from the wild populations were laid out to dry in the laboratory, and then were sorted and stored in envelopes until required.

Seeds were sown into Petri dishes, on dampened filter papers (approx. 50 per dish depending on size) and placed under light to germinate. Prior to light treatment, some seeds were given 6 weeks in the freezer at 4°C to break dormancy. The final selection of species used in this study was ultimately determined by success of germination and propagation.

Many species were sown but only a few successfully germinated and less made it to a suitable quantity for an experiment. Eventually only 14 species were used for exposure experiments (Table 2.1(a)). Table 2.1(b) show a list of the remaining 43 species collected from the six sites which could not be grown up in sufficient quantities for a viable experiment.

Following germination, seedlings were transferred to seed trays containing John Innes Seed Compost and grown for 2-3 weeks in growth chambers. The plants were then transferred on to pots (volume approx. 1.5m^3), one plant per pot, containing a potting mixture of 70% John Innes N°2 loam-based compost and 30% limestone chippings to simulate the limestone soils present in this upland habitat.

Table 2.1
List of species collected from sites within the Yorkshire Dales.
(a) Species used in exposure experiments and **(b)** other species collected.
An X indicates the sites from which the species were collected
(a)

Species	Yockenthwaite	Conistone Old Pastures	Bastow Wood	Grass Wood	Colt Park Wood	Scar Close	Malham
Anthyllis vulneria	x						
Betonica officinalis	x						
Centurea nigra						x	
Geranium lucidium					x		
Geranium robertanium		x		x		x	
Glechoma hederacea							x
Lathyrus pratensis				x	x		
Lotus corniculatus	x	x	x		x	x	
Plantago lanceolata	x	x			x		
Sanguisorba minor	x	x	x		x		
Scropularia nodosa				x			
Serratula tinctoria						x	
Solidago virgurae						x	
Valeriania officinalis				x			

Table 2.1 (b)

Species	Yockenthwaite	Conistone Old Pastures	Bastow Wood	Grass Wood	Colt Park Wood	Scar Close	Malham
Allium ursinium				x			
Angelica sylvestris						x	
Bellis perrenis	x						
Campanula latifolia					x		
Campanula rotundifolia				x		x	
Capsella bursella-pastoris		x					
Cerastium holosoides	x	x	x		x	x	
Chamerion angustifolium					x	x	
Conopodium majus	x	x			x		
Epilobium montanum		x			x		
Eriophorum vaginatum						x	
Euprasia officinalis	x				x	x	
Filipendula ulmaria				x	x	x	
Filipendula vulgaris		x					
Fragaria vesca			x	x			
Galium saxatile					x		
Geranium pratense	x				x		
Geranium sanguineum						x	
Geum rivale				x		x	
Geum urbanum		x	x	x		x	
Helianthemum nummularia	x	x	x			x	
Heracium pillosa	x	x	x		x		
Hyacinthoides non-scripta				x		x	
Hypericum pulchrum				x		x	
Hypochaeris radicata	x	x			x		
Minuartia verna	x	x	x		x	x	
Mycelis muralis						x	
Myosotis arvensis				x			
Potentilla erecta		x		x		x	
Primula farinosa			x			x	
Prunella vulgaris	x	x	x	x	x		
Rhianthus minor	x				x		
Rumex acetosa	x						
Sangusorba officinalis		x	x	x			
Scabiosa columbaria		x			x	x	

2.1 (b) Cont.

Species	Yockenthwaite	Conistone Old Pastures	Bastow Wood	Grass Wood	Colt Park Wood	Scar Close	Malham
Sussia pratense				x		x	
Taraxacum officinalis	x					x	
Thalictrum minus						x	
Trifolium dubens	x						
Trifolium pratense	x	x	x	x	x		
Trifolium repens		x	x	x	x		
Viccia cracca				x	x	x	
Viola riviana		x	x	x		x	

2.2.4 Exposure Chambers

The exposure chambers that were used in these, and subsequent, experiments are contained within an indoor room, with the dimensions 6m x 3.2m, at the University of Bradford. The fumigation chambers were eight 80cm x 80cm x 80cm Perspex boxes. Air is drawn into the fumigation system from the outside, and is filtered and air conditioned as follows: - it first passes through a pre-filter to remove particles within the air, then through a humidifier and heater, and finally through an activated charcoal filter (model SNCI SL35: Emcel Filters, Machine Control Ltd., Horsham, Sussex), fitted with an additional Purafil Filter. The air is then split between the eight chambers. The charcoal filter removes impurities from the air such as ozone and NO_2; the Purafil filter removes NO.

The chambers each receive approximately 3 air changes per minute (about 1.5 m^3 min^{-1}) of filtered air. This was regularly checked with a flow meter, and any adjustments to air flow rate were made at an individual chamber level by a valve on the incoming pipe. Each chamber also had an internal fan which kept the air

within the chambers well circulated. Air temperature and humidity are regulated by the air conditioning system. The air temperatures and humidity levels used in these experiments are given in Table 2.2. Lighting was provided by large 250W metal halide lamps (Siemens; model :HR2NJO5H), positioned centrally over the chambers. These light were on time switches to control day length (Table 2.2); photon flux density at plant level was approx 90 μmol m^{-2} s^{-1}.

Table 2.2
Temperature, Humidity and Day Length Parameters for the Exposure Chambers

	Day	Night
Time	07.00 – 19.00	19.00 -07.00
Air Temperature (°C)	22	15
Relative Humidity	80%	80%

The eight chambers are arranged in four banks of two chambers. To start with, two chambers were deemed 'nursery' chambers and were used to adjust plants to chamber conditions. These were supplied with charcoal filtered air (CFA). In these experiments only 6 chambers were used for experimental purposes. Fig 2.1 (a) shows the original arrangement of the chambers; the chambers selected for ozone treatments were those previously configured to receive ozone, which were more stable in terms of ozone levels than the others. After the installation of a new ozone generator in January 2004, it was easier to adjust and control ozone concentrations and the arrangement was

Fig 2.1
Arrangement of the eight exposure chambers **(a)** until 31/03/04 **(b)** post 31/03/04
CFA = Charcoal Filtered Air, O_3 = Ozone, N = Nursery

(a)

N	N
CFA	O_3
O_3	O_3
CFA	CFA

(b)

O_3	CFA
CFA	O_3
O_3	CFA
O_3	CFA

switched to that shown in Fig 2.1 (b). This arrangement provides four replicate blocks of the two treatments across the room. The nursery chambers were dropped as the last group of plants had time to adjust prior to fumigation commenced.

Ozone was produced by electrical discharge using a commercial generator. This was originally produced by a Wallace and Tiernan Type BA 023 ozone generator fed by an oxygen concentrator (Ozotech; Model: Power Prep 66). In Jan 2004, this was replaced by a new ozone generator (Envirorental Products; Model: PD 600). Levels of ozone in the chambers were controlled by manually operated needle valves (type MF/B/2/1/8; Platon Flowbits, Chineham, Basingstoke, Hants.)

Concentrations of ozone in the ozone treatment chambers were monitored continuously using a UV photometer (Dasibi, 1003-AH UV Photometer or Advanced Pollution Instrumentation Inc, Model API 400A). A PTFE sampling line was manually transferred between chambers so that ozone concentrations were checked hourly during the fumigations for each chamber. Regular checks were also made of ozone levels in the charcoal-filtered air (CFA) chambers.

Due to the lack of a functional data recording system, only manual records of ozone levels were made during this experiment. Because concentrations were checked and adjusted every hour by an individual, there is confidence that the target concentration was maintained as a mean over the course of this experiment. However, there was significant variation around this mean concentration depending on variability in concentrations. Up to Jan 2004, instantaneous concentrations varied typically +/-20 ppb around the target concentration. After Jan 2004, with the installation of a new generator, the variation dropped greatly,

and was rarely more than +/- 5ppb. Regular checks of the CFA chambers revealed that ozone concentrations varied between 1-5ppb.

Procedures were followed to ensure the quality of the ozone concentration data. Routine checks were made of the span and zero values, and the internal calibration unit was used to check the calibration of both monitors at the start of the experiment, in September 2004 and in January 2005. The calibration unit of the Dasibi analyser was checked in July 2004 against a monitor pre-checked against an external standard by Dr M Coyle, Centre for Ecology and Hydrology Edinburgh) and the calibration was within +/-1%.

2.2.5 Experimental Design

Plants were first selected from the stock of seedlings and divided into groups for each fumigation chamber. The groups were carefully selected in order to ensure that each chamber's plants were of a similar size and maturity, although in some cases this meant that each group comprised various sized plants. They were then transferred to the nursery exposure chambers for 1 week to adjust to conditions.

The plants were then distributed into eight or six chambers; half of the chambers were supplied with charcoal filtered air (CFA) and half were supplied with a target concentration of 80ppb ozone for 8 hours a day. 80ppb of ozone was selected due to it being two-times the dose of the AOT40, and would therefore give a quick differentiation between sensitive and resistant plants. Fumigation periods were 15 days with ozone exposure, although the period over which these were supplied varied from 15 to 20 days (see Table 2.3). The plants were maintained in a moist soil with regular watering.

2.2.6 Experimental design and plant response measurements

Table 2.3 summarises all the information concerning the exposure studies for each species, including the number of individual plants, dates of study, duration of exposure, experimental parameters and ecological parameters. Recorded plant growth parameters measured before and after the exposure period are given in Table 2.4. Leaf and root biomass and visible injury were recorded for all species, but other measurements were species-specific and depended on the species' natural growth form. Prior to fumigation of the plants, the leaf number and other growth parameters were recorded, as appropriate for the species (see Table 2.4). These growth parameters were measured partly in order to track changes in growth of the plant over the fumigation period (see Table 2.4), and partly to provide an initial measurement of variation between and within chambers for use in analysis of covariance. A short description of how each of the parameters was measured is given in Table 2.5.

Table 2.3
Summary of Experimental dates for each species and population including, duration of exposure and experimental parameters .

Species	Site Of Collection	Fumigation Period	Number of chambers	Number of Plants	Plants per chamber	Number of days in Chambers	Number of fumigation days	AOT40 (ppb h-1)
Anthyllis vulneria	Yockenthwaite meadows	03/12/2003 -19/12/2003	6	48	8	16	15	4800
Betonica officinalis	Yockenthwaite meadows	08/05/2004 -23/05/2004	8	31	3/4	15	15	4800
Centaurea nigra	Scar Close	12/11/2003 -03/12/2003	6	30	5	21	15	4800
Geranium lucidum	Ribblehead	11/03/2004 -30/03/2004	6	48	8	15	15	4800
Geranium robertainium	Scar Close	11/03/2004 -30/03/2004	6	48	8	15	15	4800
Glechoma hederacea	Malham	13/03/2004 -30/03/2004	6	24	4	17	15	4800
Lathyrus pratensis	Ribblehead	25/11/2003 -13/12/2003	6	41	7/8	18	15	4800
Lotus corniculatus	Conistone Old pastures	03/12/2003 -19/12/2003	4	16	4	16	15	4800
Lotus corniculatus	Bastow Wood	03/12/2003 -19/12/2003	6	48	8	16	15	4800
Lotus corniculatus	Ribblehead	03/12/2003 -19/12/2003	4	17	4/5	16	15	4800
Lotus corniculatus	Scar Close	03/12/2003 -19/12/2003	6	48	8	16	15	4800
Plantago lanceolata	Conistone Old pastures	10/03/2004 -30/03/2004	6	48	8	15	15	4800
Plantago lanceolata	Yockenthwaite meadows	10/03/2004 -30/03/2004	6	40	6/7	15	15	4800
Sanguisorba minor	Conistone Old pastures	13/06/2004 -28/06/2004	8	48	6	15	15	4800
Sanguisorba minor	Bastow Wood	13/06/2004 -28/06/2004	8	48	6	15	15	4800
Sanguisorba minor	Ribblehead	13/06/2004 -28/06/2004	8	48	6	15	15	4800
Scrophularia nodosa	Grass Wood	13/06/2004 -28/06/2004	8	32	4	15	15	4800
Serratula tinctoria	Scar Close	05/04/2004 -17/04/2004	6	48	8	12	12	3840
Solidago virgireau	Scar Close	05/04/2004 -17/04/2004	6	27	4/5	12	12	3840
Valeriania officinalis	Grass Wood	02/02/2004 -23/02/2004	6	66	11	21	15	4800

Table 2.4
Summary of experimental parameters measured prior to, during and post exposure; X indicates measured indices.

Species	Site Of Collection	Initial Measurements					Biomass					Leaf Number	Leaf Area	Leaf Length	Leaf Width	Stem Length	Number of Stems	Phenotypic Variation	Stomatal conductance	Dead Leaves	Visible Damage
		Leaf Number	Stem Length	Number of Stems	Biomass	Leaf Length	Upper				Roots										
							Leaves	Buds	Stems	Flowers											
Anthyllis vulneria	Yockenthwaite meadows	X	-	-	-	-	X	-	-	-	X	X	X	X	-	-	-	-	-	X	X
Betonica officinalis	Yockenthwaite meadows	X	-	-	-	-	X	-	-	-	X	X	X	-	-	-	-	-	-	X	X
Centaurea nigra	Scar Close	X	-	-	-	-	X	-	-	-	X	X	X	-	-	-	-	-	-	X	X
Geranium lucidum	Ribblehead	X	-	-	X	-	X	-	-	-	X	X	X	-	-	-	-	-	X	X	X
Geranium robertainium	Scar Close	X	-	-	X	-	X	-	-	-	X	X	X	-	-	-	-	-	-	X	X
Glechoma hederacea	Malham	X	X	X	-	X	X	-	X	-	X	X	X	X	-	X	X	-	X	X	X
Lathyrus pratensis	Ribblehead	X	X	X	-	-	X	-	X	-	X	X	-	-	-	X	X	-	-	X	X
Lotus corniculatus	Conistone Old pastures	X	X	X	-	-	X	-	-	-	X	X	X	-	-	X	X	-	-	X	X
Lotus corniculatus	Bastow Wood	X	X	X	-	-	X	-	-	-	X	X	X	-	-	X	X	-	-	X	X
Lotus corniculatus	Ribblehead	X	X	X	-	-	X	-	-	-	X	X	X	-	-	X	X	-	-	X	X
Lotus corniculatus	Scar Close	X	X	X	-	-	X	-	-	-	X	X	X	-	-	X	X	-	-	X	X
Plantago lanceolata	Conistone Old pastures	X	-	-	X	-	X	-	-	-	X	X	X	X	X	-	-	X	-	X	X
Plantago lanceolata	Yockenthwaite meadows	X	-	-	X	-	X	-	-	-	X	X	X	X	X	-	-	X	-	X	X
Sanguisorba minor	Conistone Old pastures	X	-	-	X	X	X	-	-	-	X	X	X	-	-	-	-	-	-	X	X
Sanguisorba minor	Bastow Wood	X	-	-	X	X	X	-	-	-	X	X	X	-	-	-	-	-	-	X	X
Sanguisorba minor	Ribblehead	X	-	-	X	X	X	-	-	-	X	X	X	-	-	-	-	-	-	X	X
Scrophularia nodosa	Grass Wood	X	-	-	-	-	X	X	X	X	X	X	X	-	-	X	-	-	X	X	X
Serratula tinctoria	Scar Close	X	-	-	X	X	X	-	-	-	X	X	X	X	-	-	-	-	X	X	X
Solidago virgireau	Scar Close	X	-	-	-	-	X	-	-	-	X	X	X	X	-	-	-	-	-	X	X
Valeriania officinalis	Grass Wood	X	-	-	X	X	X	-	-	-	X	X	X	X	-	-	-	-	X	X	X

2.2.5 Stomatal Conductance

Stomatal conductance was measured on those plants whose leaves were large enough for the requirements of the infra-red gas analyser (Cirras I, PP-systems). Measurements were made between 12.00 and 13.00 and for some species, night-time measurements were made between 23.00 and 24.00. Ozone was present during the day-time measurements, but the plants were removed from the chambers during the time they were measured. Each plant was measured for 2 minutes at 20 second intervals, and the mean value was taken. These plants had a leaf tagged for this purpose and conductance was measured before the start of the fumigation period, in the middle, and at the end of the fumigation period.

2.2.6 Statistical analysis

Statistical analysis was carried out in SPSS (Version 14, SPSS Inc.). All data for individual plants, across replicate chambers, were collated into two treatment groups; either 'ozone' or 'control', the data for all parameters were then tested for ozone effects using a one-way analysis of variance. When ozone treatment effects were significant, the data were analysed also with one-way ANOVA to test for chamber effects. However, there were no significant chamber effects detected for this experiment. This provides the justification for pooling the data for individual plants. Data was not transformed and a normal distribution was assumed; all of the data met conditions for a normal distribution.

Initial measurements, as given in Table 2.4, were used as a covariate for the ANOVA for specific growth indices. However, the effect of the covariate was only significant at $P = 0.05$ in two cases: - the Conistone Old Pastures population of *Lotus*

corniculatus and the Ribblehead population of *Sanguisorba minor*. This is noted in the text in the results section.

Table 2.5
Description of the methods used for each measured parameter.

Measured Parameter	Description and Method
Biomass	The parts of the plant were carefully separated and the roots were washed in water to remove soil. They were then left in an oven to dry at 100°C for 48 hours, left to cool in a desiccator. When the specimens were dry they were then weighed
Dead Leaves	The number of dead leaves per plant.
Leaf Area	Leaf area (cm^2) measured using a leaf area machine.
Leaf Length	The length (cm) of the leaf from the petiole or base of leaf to the leaf tip.
Leaf Number	A count of the total number of leaves per plant.
Leaf Width	The leaf width at the observed widest point (cm)
Number of Stems	The total number of stems arising from the central base of the plant
Phenotypic Variation	For *P.lanceolata* recorded at the end of the experiment for different types of phenotype. See discussion for further details.
Stem Length	The length (cm) of the stem from the base of the plant to the longest growing tip

Signs of visible foliar stress and damage were only observed on the following species: *Geranium lucidium*, *Centurea nigra, Glechoma hederacea* and *Scrophularia nodosa*. All plants of these species were assessed periodically, and at the end of the experiment, for ozone specific injury (in the form of white or yellow stipples on the leaf surface), and for evidence of foliar injury or stress (e.g. increased reddening of the leaf) and senescence. Measurements were recorded as percentage injury of the individual leaf and averaged per plant. A leaf was classified as senesced if 25% or more of the leaf was senesced.

2.3 Results

2.3.1 Introduction

A summary of all the response variables measured at the end of the fumigation period is given in Table 2.6 together with the results of ANOVA. Eight species showed a significant response to ozone treatment in at least one variable, while five species: - *B.officinalis*, *C.nigra, L.pratensis*, *S.virgireau* and *S.tinctoria* showed no significant response. The other seven species showed no significant effect on any variable.

Three of the four populations of *Lotus corniculatus* showed a negative response to ozone treatment; the Scar Close population showed only one response and this was a positive effect on leaf area. Both populations of *Plantago lanceolata* showed responses to ozone and all three populations of *Sanguisorba minor* showed responses to treatment with ozone. None of the species/populations obtained from Scar Close showed any negative response to ozone fumigation. The results for each of the seven species with significant treatment effects are discussed in turn below.

Table 2.6

Summary of Results from the Fumigation of 14 Species from the Yorkshire Dales National Park.

*NS = Non significant response, ↓= decrease under ozone, ↑= increase under ozone, - indicates no measurement made; 'None' indicates no evidence of visible injury or foliar stress; * = P < 0.10, ** = P< 0.05, *** = P < 0.01.*

		Biomass				Leaf Number	Leaf Area	Leaf Length	Leaf width	Stem Length	Number of Stems	Stomatal conductance	Dead Leaves	Visible Damage
		Above Ground	Roots	Total	R:S									
Anthyllis vulneria	Yockenthwaite meadows	NS	NS	NS	↓*	NS	-	NS	-	-	-	-	-	-
Betonica officinalis	Yockenthwaite meadows	NS	NS	NS	NS	NS	NS	-	-	-	-	-	-	-
Centurea nigra	Scar Close	NS	NS	NS	NS	NS	NS	-	-	-	-	-	NS	NS
Geranium lucidium	Colt Park Wood	NS	↓**	NS	↓***	NS	↓***	-	-	-	-	↑***	NS	↑***
Geranium robertainium	Scar Close	NS	NS	NS	NS	NS	NS	-	-	-	-	-	NS	-
Glechoma hederacea	Malham	NS	NS	NS	NS	NS	↓**	↓**	-	NS	NS	NS	NS	↑***
Lathyrus pratensis	Colt Park Wood	NS	NS	NS	NS	NS	-	-	-	NS	NS	-	NS	NONE
Lotus corniculatus	Conistone Old pastures	↓**	↓**	↓**	NS	↓*	-	-	-	NS	↓*	-	-	NONE
Lotus corniculatus	Bastow Wood	NS	↓***	NS	↓***	NS	-	-	-	NS	NS	-	-	NONE
Lotus corniculatus	Colt Park Wood	NS	NS	NS	NS	NS	-	-	-	NS	NS	-	-	NONE
Lotus corniculatus	Scar Close	NS	NS	NS	NS	NS	-	-	-	NS	NS	-	-	NONE
Plantago lanceolata	Conistone Old pastures	NS	NS	NS	NS	NS	NS	NS	↓**	-	-	-	NS	NONE
Plantago lanceolata	Yockenthwaite meadows	NS	NS	NS	NS	NS	NS	NS	↓**	-	-	-	NS	NONE
Sanguisorba minor	Conistone Old pastures	NS	-	-	-	NS	↑*	-	-	-	-	-	↑*	NONE
Sanguisorba minor	Bastow Wood	NS	-	-	-	NS	↑*	-	-	-	-	-	↓**	NONE
Sanguisorba minor	Colt Park Wood	NS	-	-	-	↑*	NS	-	-	-	-	-	↑*	NONE
Scrophularia nodosa	Grass Wood	NS	NS	NS	NS	NS	NS	-	-	-	-	↓**	-	↑***
Serratula tinctoria	Scar Close	NS	NS	NS	NS	NS	NS	NS	-	-	-	NS	-	NONE
Solidago virgireau	Scar Close	NS	NS	NS	NS	NS	-	NS	-	-	-	-	-	NONE
Valeriania officinalis	Grass Wood	NS	NS	NS	↓*	NS	NS	NS	-	-	-	↓*	-	NONE

2.3.2 *Anthyllis vulnaria*

Ozone exposure resulted in a decrease of 53% in the root / shoot ratio ($F = 3.32$; $P < 0.10$) (Fig 2.2). There were no other significant effects of ozone on *A.vulneria*.

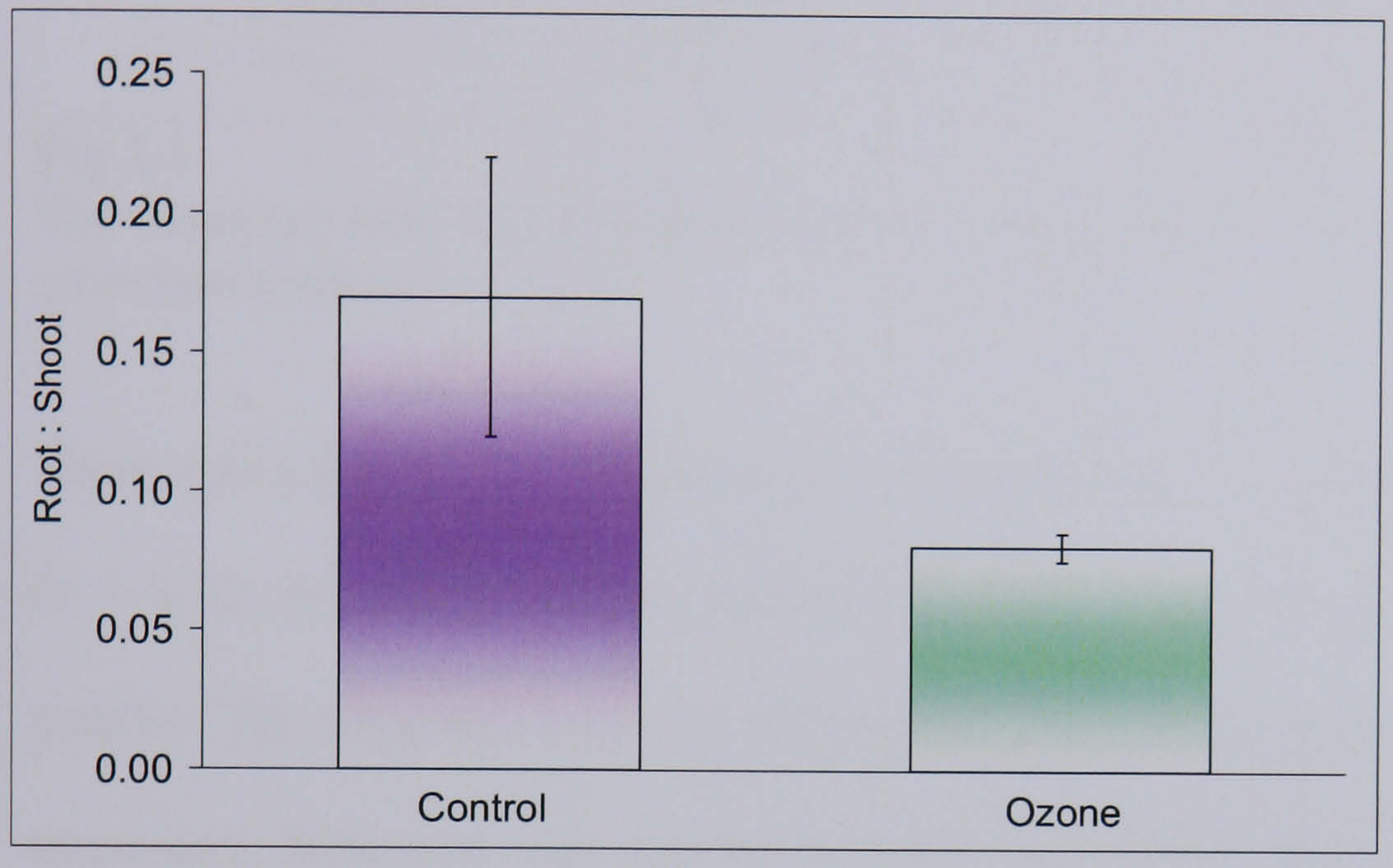
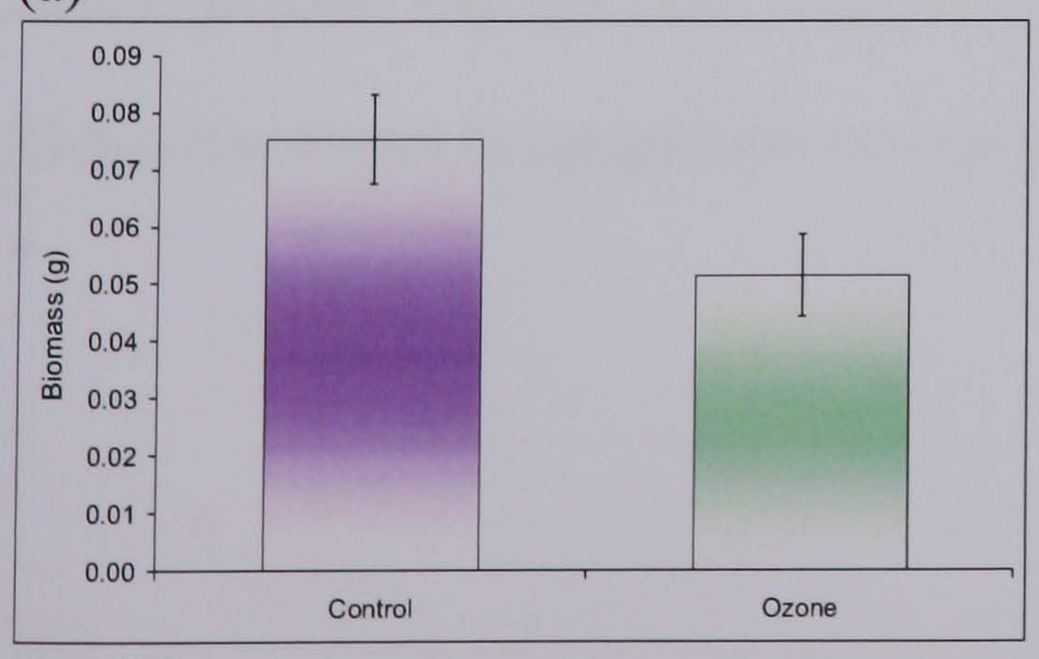


Fig 2.2
The mean root / shoot ratio in the two treatments for *Anthyllis vulneria* : error bars indicate +/- 1.s.e.

2.3.2 *Geranium lucidium*

In ozone, *G.lucidium* had a significant reduction in root biomass of 32%, ($F=5.09$; $p<0.05$) (Fig 2.3). There was also a reduction in root shoot ratio of 34% ($F=10.45$; $p<0.05$). However there was no significant difference in total biomass or above ground biomass.

(a) (b)



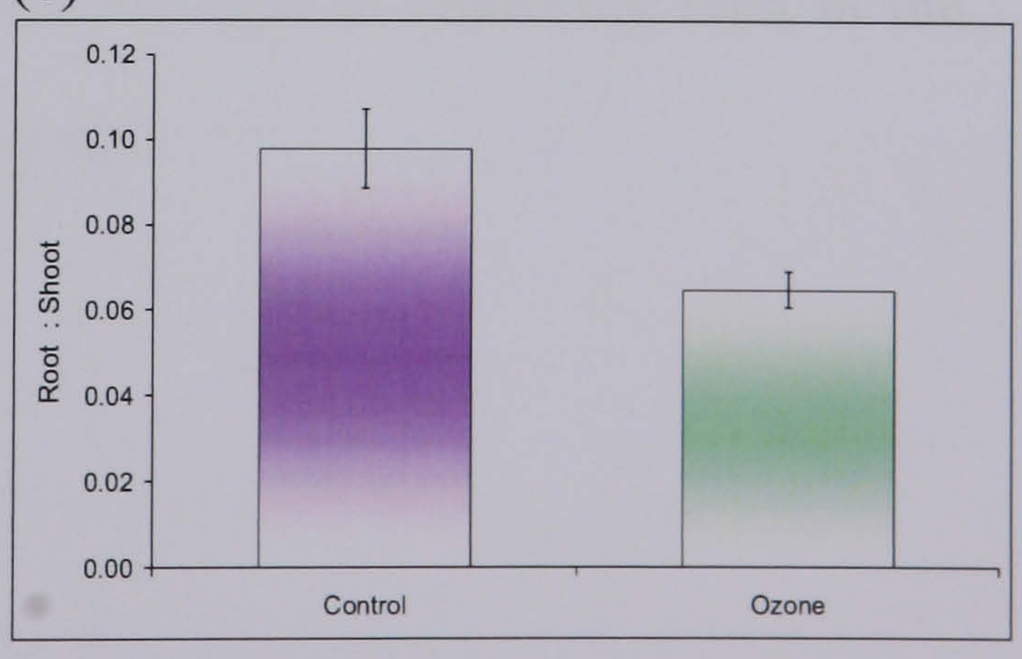


Fig 2.3
Mean (a) root biomass (g) and (b) root / shoot ratio in the two treatments for *G.lucidum*: error bars indicate +/- 1s.e.

Original in colour

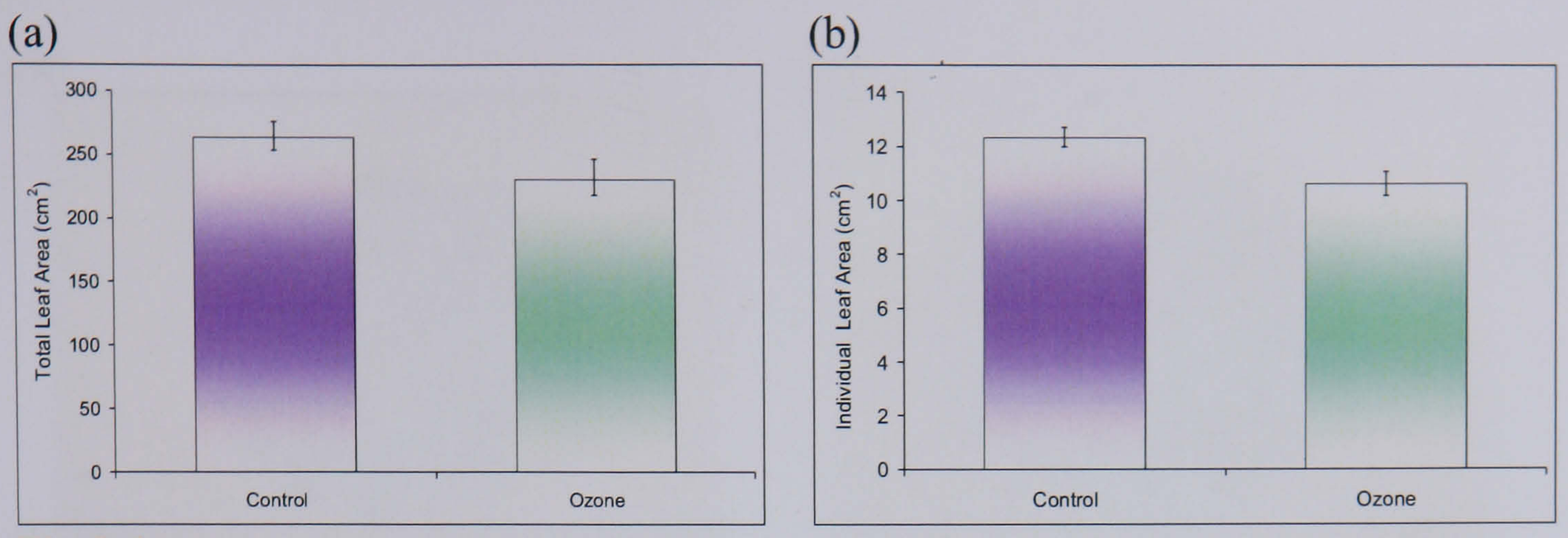


Fig 2.4
The mean (a) total leaf area (cm^2) and (b) individual leaf area (cm^2) for *G.lucidium:* error bars indicate +/- 1s.e.

There was a significant difference between treatments in mean individual leaf area ($F = 8.92$; $p<0.01$) (Fig 2.4); individual leaves in the ozone treatment were 8% smaller. The total leaf area was reduced by a similar amount (11%) in the ozone treatment; however, due to the greater variability, this difference was not significant at the 5% level ($F = 2.73$; $p=0.106$).

Although there were no specific ozone symptoms, the *G.lucidium* plants in the ozone treatment showed signs of stress by increased reddening of the leaves whereas, in general, the individuals in the control treatment had bright green healthy looking leaves. The reddening of leaves, which is most likely related to increased anthocyanin levels, was significantly greater under ozone treatment ($F = 10.08$; $p<0.01$; Fig 2.6 (a)). Fig 2.5 shows the type of reddening seen in the *G.lucidum* leaves in comparison to a healthy leaf.

Original in colour

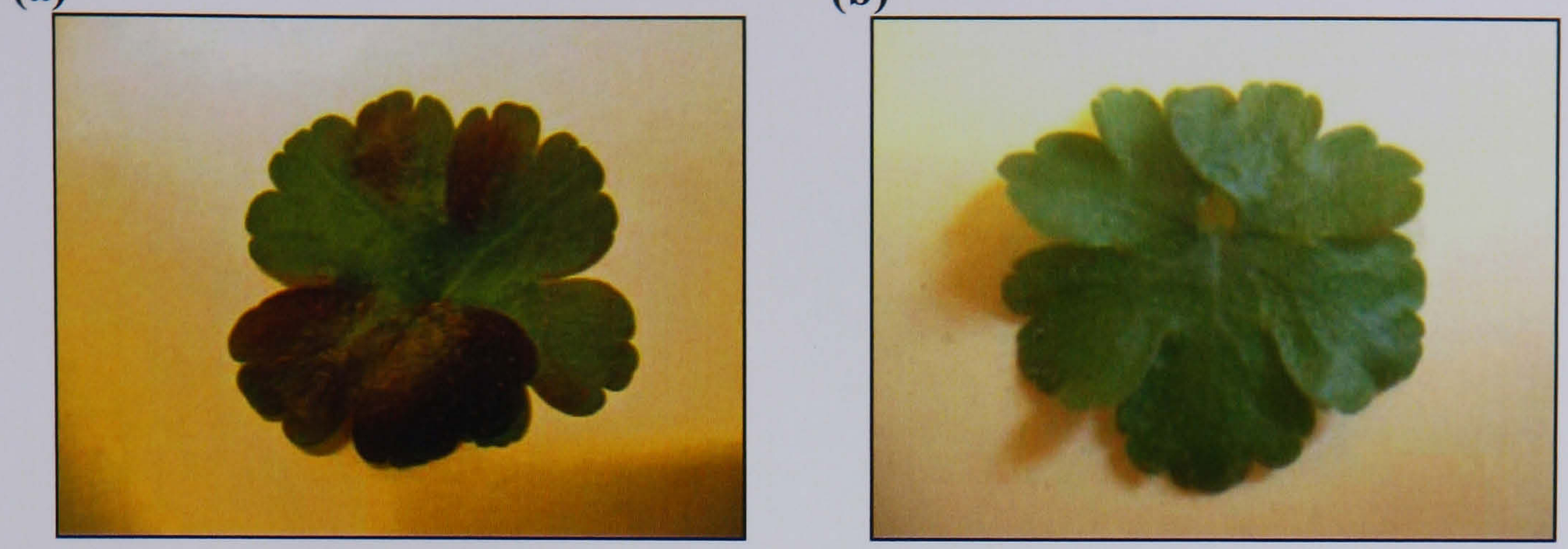


Fig 2.5
Leaves of *G.lucidium* showing (a) reddening and (b) a healthy leaf

Of the leaves that were showing foliar stress, the degree of leaf damage was estimated by categorising them into leaves that showed 1-30%; 31-70%; and 70-100% of the leaf area showing the red pigmentation. Fig 2.6 (b) shows the mean number of leaves within each category per treatment. For these leaves, there was a significant difference between treatments in the degree of damage to the leaves. There was a higher number of damaged leaves overall in the ozone treatment. Although the number of leaves showing little damage (< 30%) and severe damage (>70%) did not differ significantly between treatments, the number of leaves showing intermediate levels of damage (30-70% of their surface damaged) more than doubled in ozone ($F = 12.98$; $p < 0.01$).

Original in colour

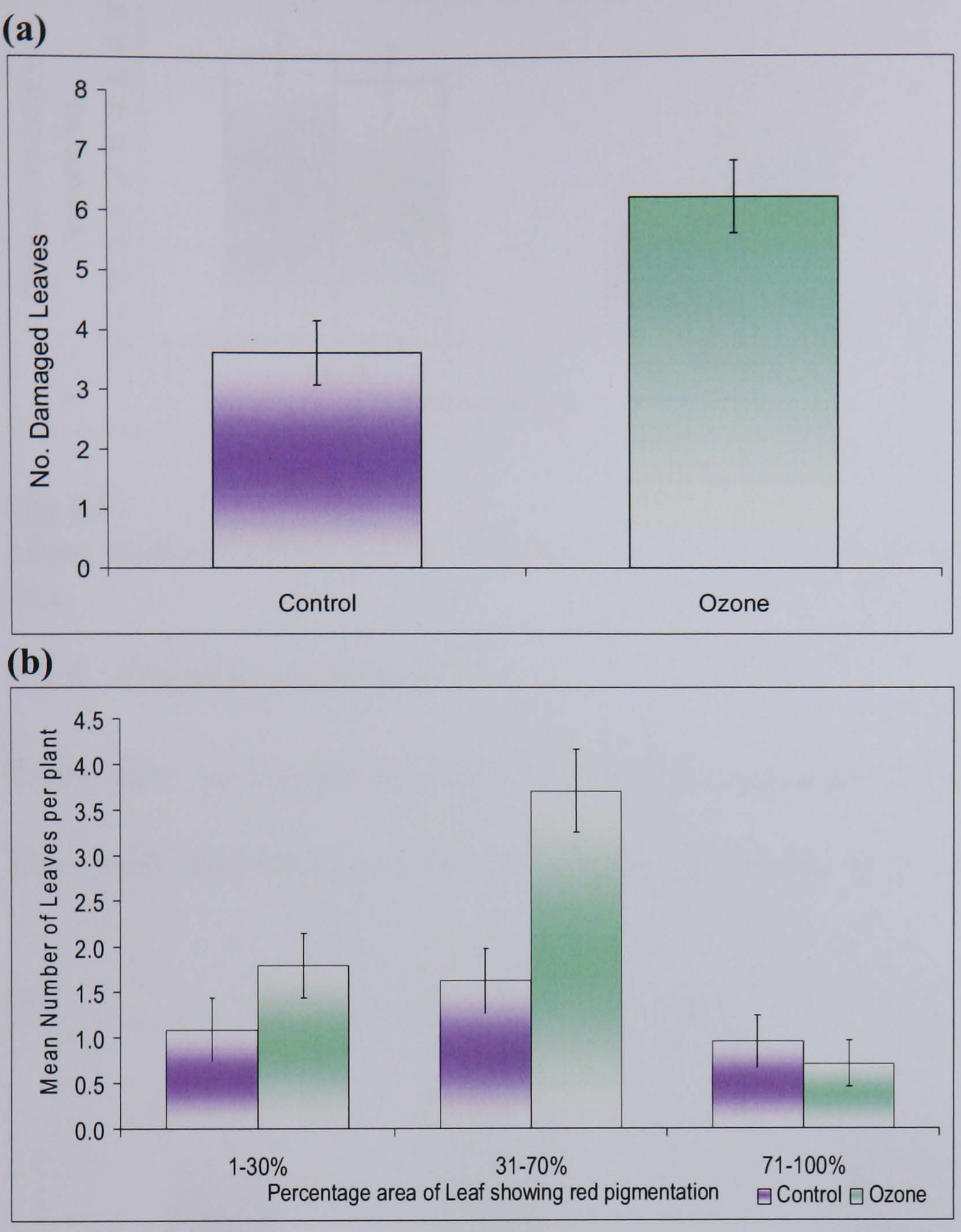


Fig 2.6
The mean (a) number of leaves per plant showing damage and (b) the mean degree of damage for *G.lucidum*: error bars indicate +/- 1.s.e.

Stomatal conductance was measured on the youngest leaf at 10 days and the same leaf again at 20 days at the end of the experiment. At 10 days, there was no significant difference between treatments, but at 20 days, stomatal conductance was significantly higher in the ozone treatment ($F = 11.07$; $p < 0.01$) (Fig 2.7).

Original in colour

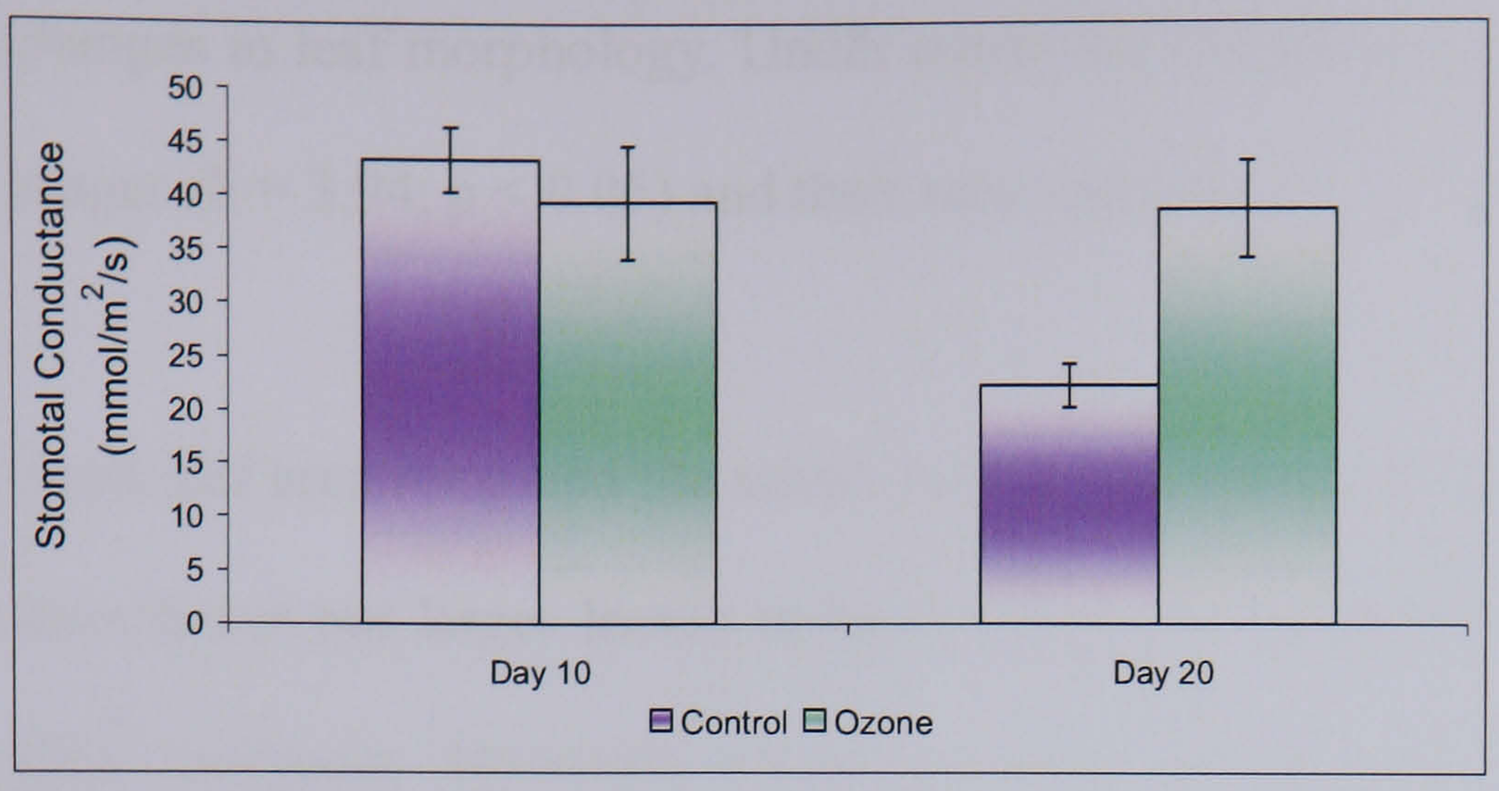


Fig 2.7
Mean stomatal conductance (mmol m^{-2} s^{-1}) for *G.lucidum*: error bars indicate +/- 1s.e.

2.3.3 Glechoma hederacea

There were no significant effects on total or component biomass in this species.

The most interesting significant effects of ozone on *G.hederacea* were the

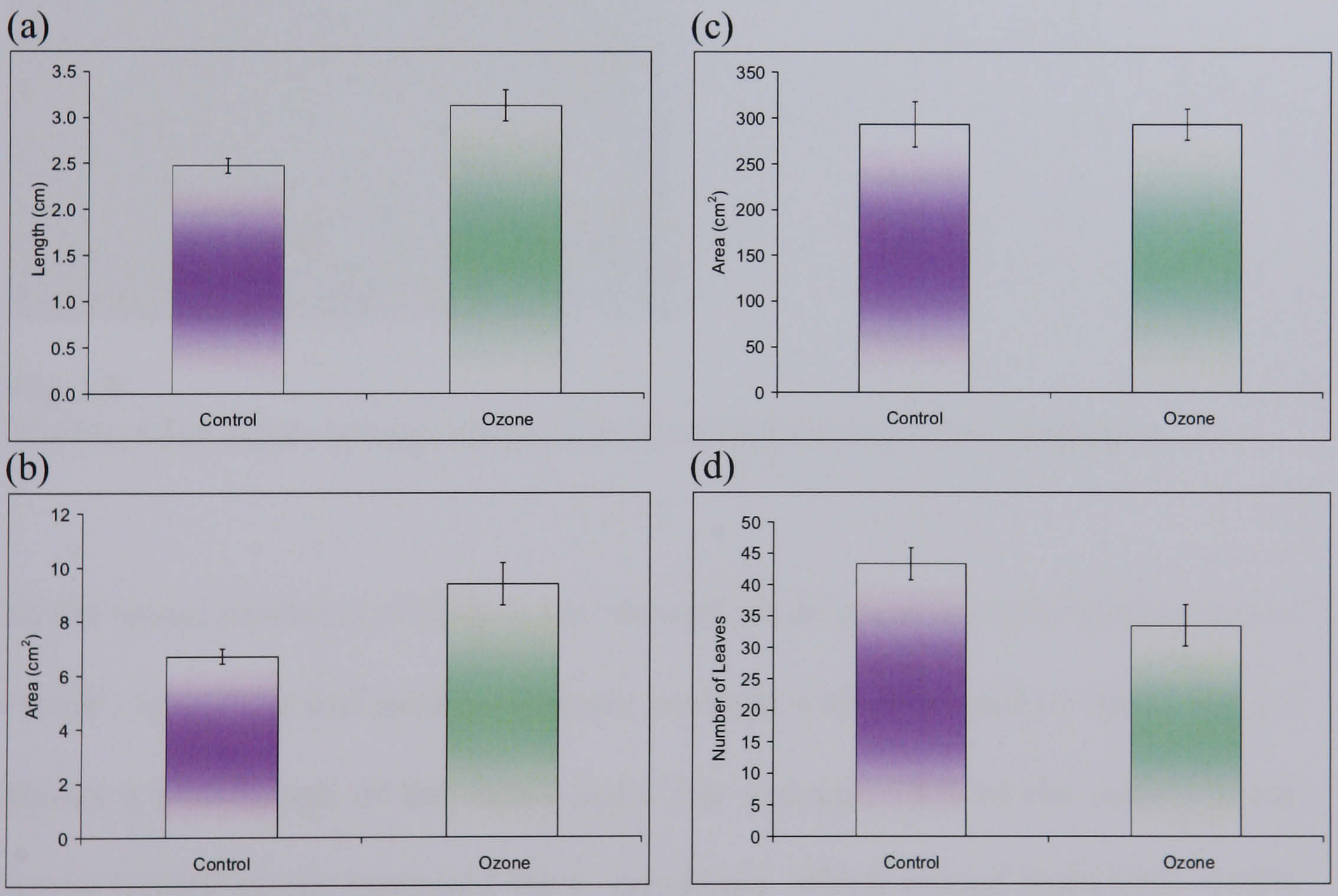


Fig 2.8
The mean (a) leaf length (cm); (b) individual leaf area (cm^2); (c) total leaf area (cm^2) and (d) number of leaves per plant for both the control and ozone treatment of *G.hederacea* : error bars indicate +/- 1s.e.

changes in leaf morphology. Under ozone the individual leaves were significantly longer ($F = 3.94$; $p < 0.05$) and their area was larger ($F = 2.83$; $p < 0.05$) (Fig 2.8).

Total leaf area remained the same, on average, between treatments, because there were fewer but larger leaves in the O_3 treatment, but many smaller leaves in the CFA treatment. However, effects of ozone on both these variables were not significant. In summary, the data suggest that ozone caused *G.hederacea* to have fewer larger leaves.

Fig 2.9
Visible foliar injury symptoms on *G.hederacea* leaves in ozone treatment

In the ozone treatment *G.hederacea*, showed ozone specific injury in the form of 'fleck', where the leaf surfaces become covered with small yellow spots. Fig 2.9 shows a photograph of the injury seen. On average, 14% of the leaves in the ozone treated group expressed these symptoms, which ranged from 20% -100% coverage of the leaf surface. As this injury was not seen in the control treatment the effect is highly significant ($F = 59.24$; $p < 0.01$).

2.3.4 *Lotus corniculatus*

There were four populations of *L.corniculatus*, results for which are presented in turn here. Only two populations showed significant responses to ozone; these were the populations from Conistone Old Pastures and Bastow Wood. However all populations, independent of treatment, were distinctly different in terms of growth and size. The two populations which showed response to exposure are considered in turn below.

2.3.4.1 Conistone Old Pastures (COP)

Ozone exposure resulted in a 12% reduction in leaf number ($F = 3.71$; $p < 0.10$) and a 8% decrease in stem number ($F = 3.86$; $p < 0.10$) midway through the fumigation period. However, by the end of the experiment there was no longer a significant difference between treatments for either index. Using the initial measurements of both indices as a covariate, differences in leaf number were still significant at this midpoint ($F = 5.368$; $p < 0.05$) but those in the number of stems were not. From the start of the fumigation period and through the experiment, the ozone treatment had a lower mean value for both leaf number and the number of stems (Fig 2.10).

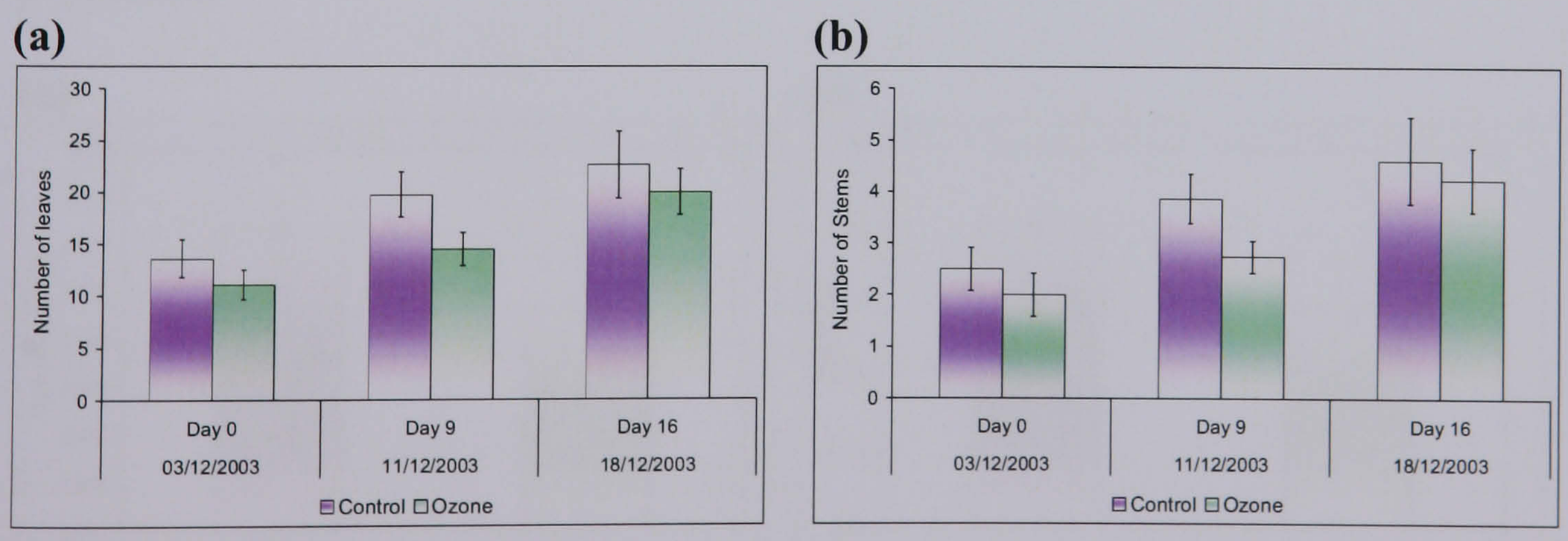


Fig 2.10
The mean (a) number of leaves per plant and (b) number of stems per plant of *L.corniculatus* population from Conistone Old Pasture: error bars indicate +/- 1s.e.

Both the above ground ($F = 5.03$; $p < 0.05$) and below ground biomass ($F = 6.33$; $p < 0.05$) were significantly reduced in the ozone treatment, by 32% and 46% respectively, and there was a consequential significant reduction in total biomass of 35% ($F = 7.98$; $p < 0.05$) (Fig 2.11).

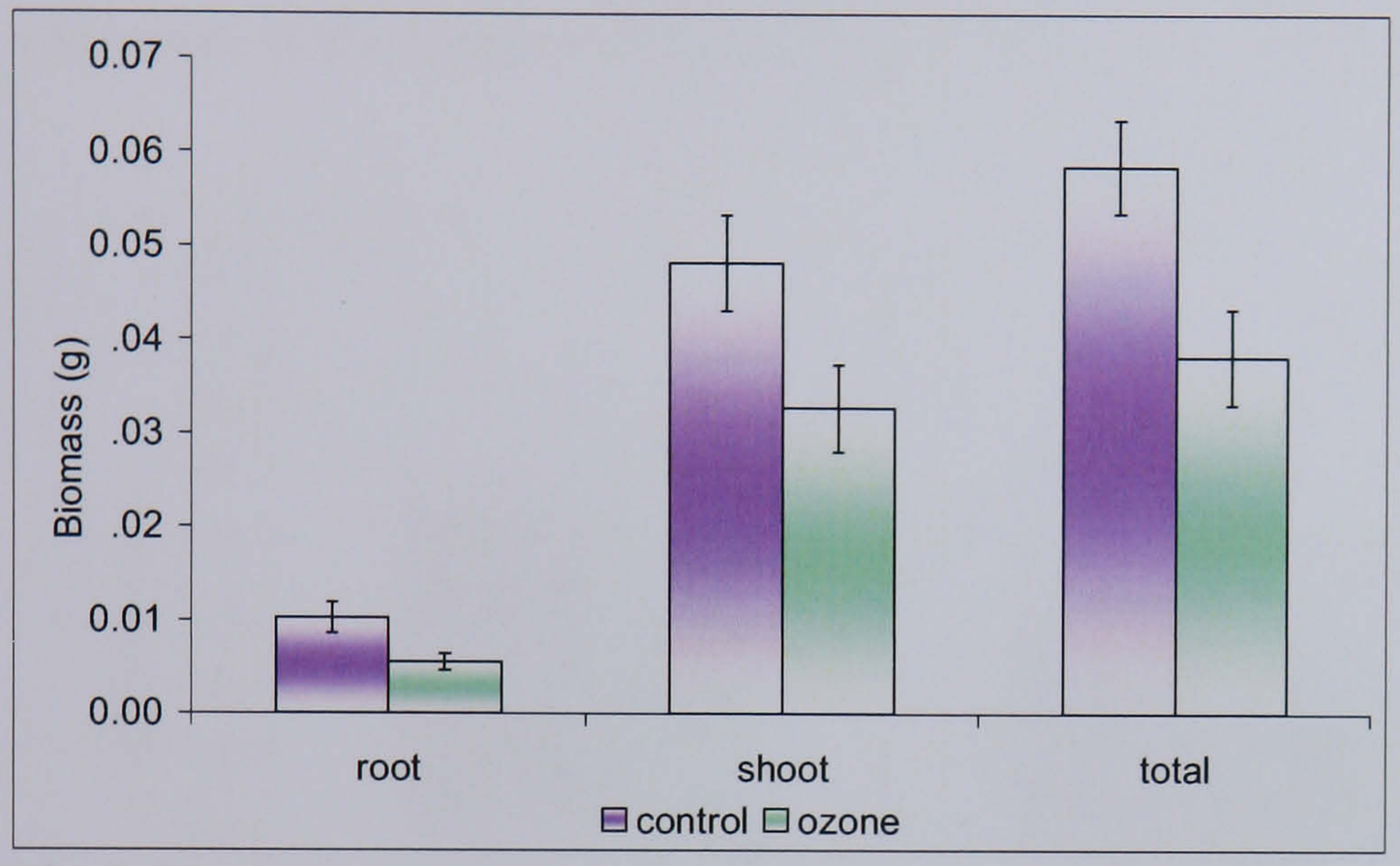


Fig 2.11
The mean live biomass (g) of *L.corniculatus* population from C.O.P: error bars indicate +/- 1.s.e

2.3.4.2 Bastow Wood (BW)

The BW population of *L.corniculatus* had a significantly reduced root biomass in ozone by 36% ($F = 4.83$; $p < 0.05$) and in the root / shoot ratio by 39% ($F = 4.62$; $p < 0.05$) (Fig. 2.12). There were no other significant changes in ozone for this population.

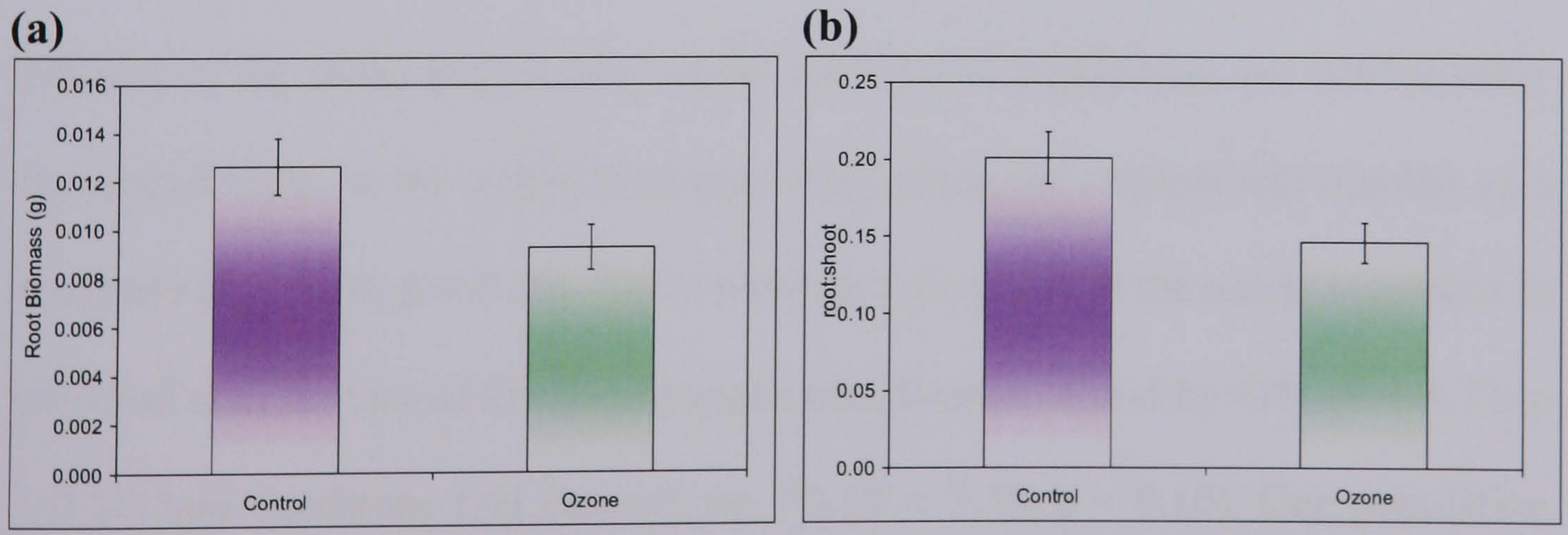


Fig 2.12
Mean **(a)** root biomass (g) and **(b)** root:shoot ratio of *L.corniculatus* population from Bastow Wood: error bars indicate +/- 1s.e.

Original in colour

2.3.5 *Plantago lanceolata*

The two populations of *P.lanceolata* came from meadow communities, one from Yockenthwaite Meadows and one from Conistone Old Pastures. Both populations of *P.lanceolata* showed no significant effects of ozone on any biomass parameters. However under ozone both populations of *P.lanceolata* showed a significant reduction in the width of the leaves ($F = 4.36$ and 5.14; $p < 0.05$) (Fig 2.13).

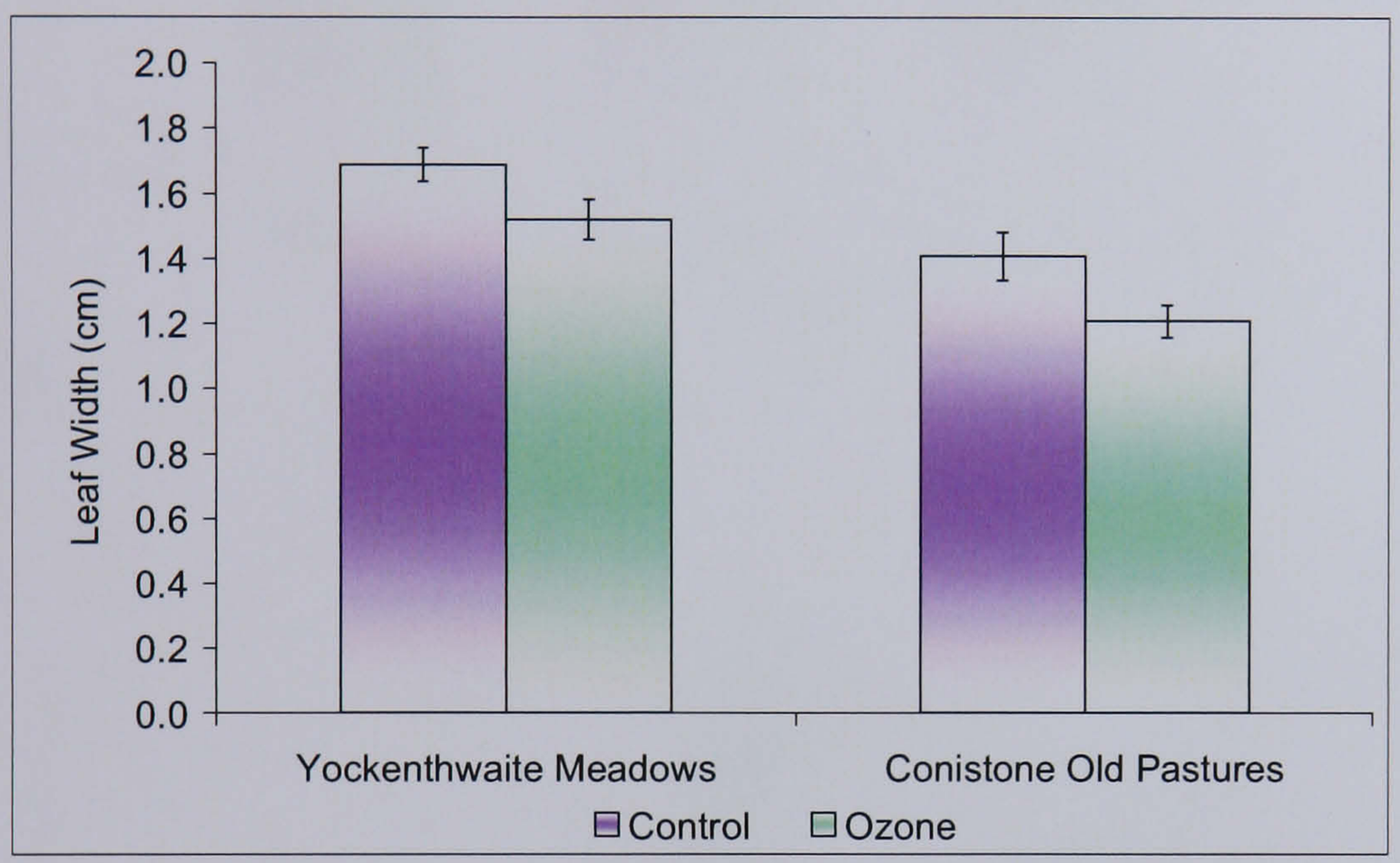


Fig 2.13
The mean leaf width (cm) of *P.lanceolata* from two populations: error bars indicate +/- 1s.e.

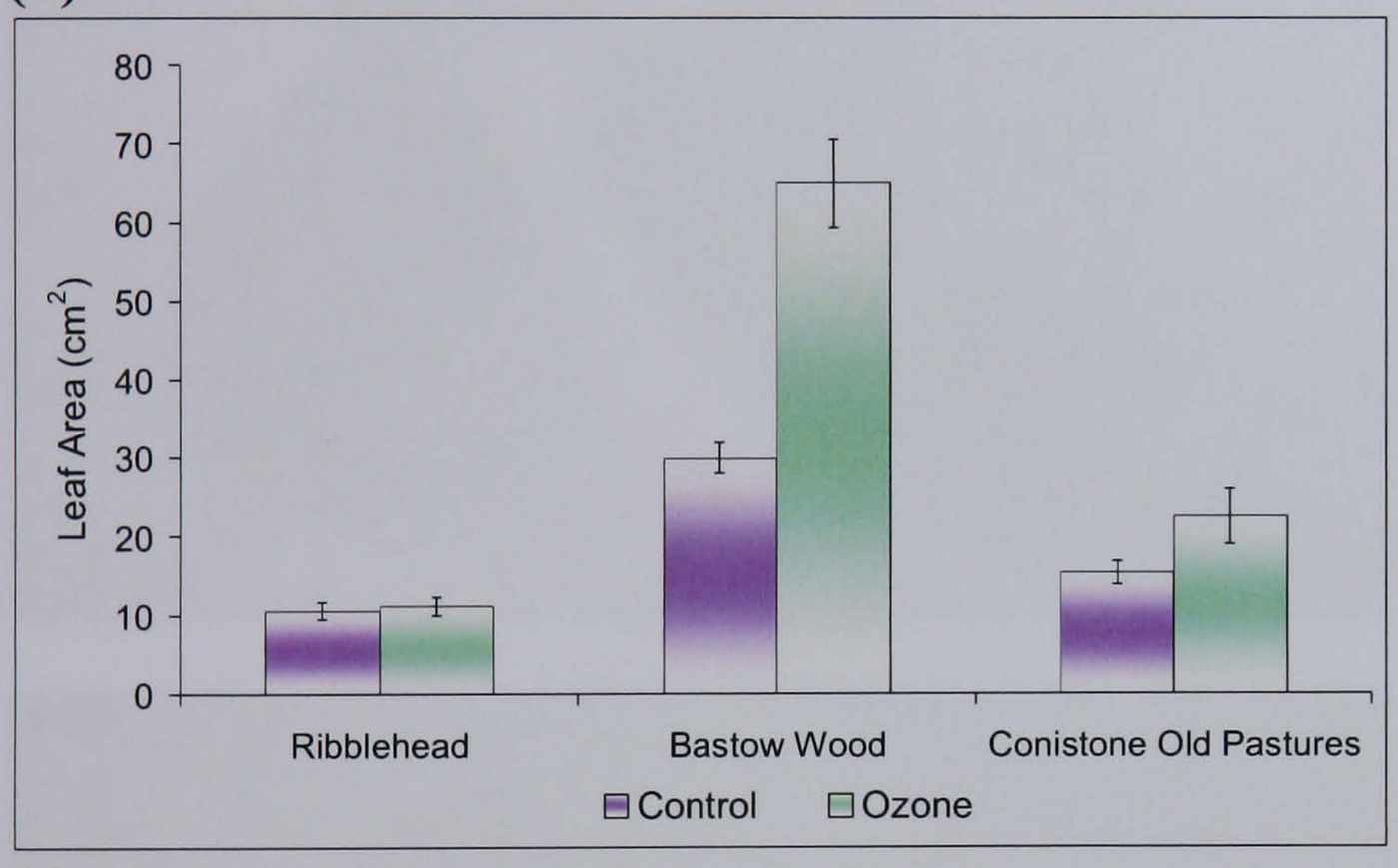
2.3.6 *Sanguisorba minor*

The three populations of *S. minor* showed positive responses to ozone, with increases in leaf number and leaf area (Fig 2.14 (a) and (b)). There was a small increase in the mean leaf number of the Ribblehead population, but not the other two populations, in the ozone treatment when using the original leaf number as a covariate ($F = 3.34$; $p < 0.10$). There were also increases in the ozone treatment in total leaf area for two of the three populations, Bastow Wood by 47% ($F = 3.77$; $p < 0.10$) and Conistone Old Pastures by 7% ($F = 3.38$; $p < 0.10$). One population showed increases in the number of dead leaves in ozone (Fig. 2.14 (c)). Conistone

Old Pastures ($F = 3.03$; $p < 0.10$) both showed a significant 65% increase in the number of dead leaves in ozone at the end of the fumigation period.

(a)

(b)



(c)

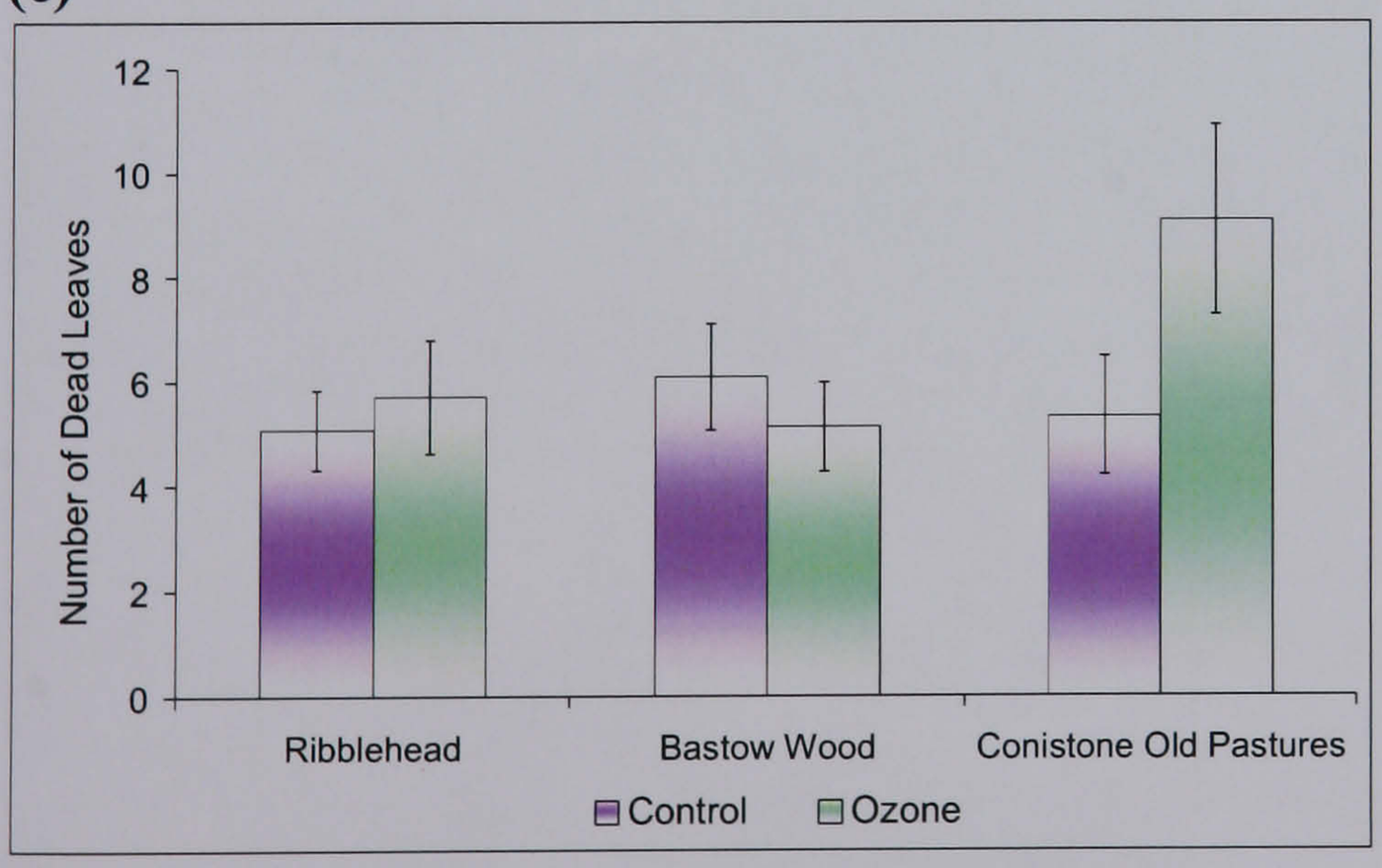


Fig 2.14
The mean (a) leaf number, (b) leaf area (cm^2) and (c) number of dead leaves, of three populations of *S.minor*: error bars indicate +/- 1s.e.

Original in colour

2.3.7 *Scropularia nodosa*

Scropularia nodosa showed ozone specific symptoms of visible foliar injury to the leaves in the ozone treatments; 88% of the plants showed these symptoms, ranging from 8% - 50% coverage of the total leaf surfaces. These flecking symptoms (Fig 2.15) were only seen in the ozone fumigated plants, making the treatment effect highly significant ($F = 23.38$; $P < 0.01$).

Fig 2.15
Scrophularia nodosa leaves showing the form of leaf fleck

Original in colour

S.nodosa showed significantly reduced stomatal conductance in the ozone treatment ($F = 6.07$; $P < 0.05$) (Fig. 2.16). Prior to any fumigation, recordings of stomatal conductance were taken from the ozone treatment group; these values are higher than those taken midway through the fumigation period ($F = 5.42$; $p < 0.10$). Stomatal conductance was also recorded at night, when there was no significant difference between treatments (Fig. 2.17a).

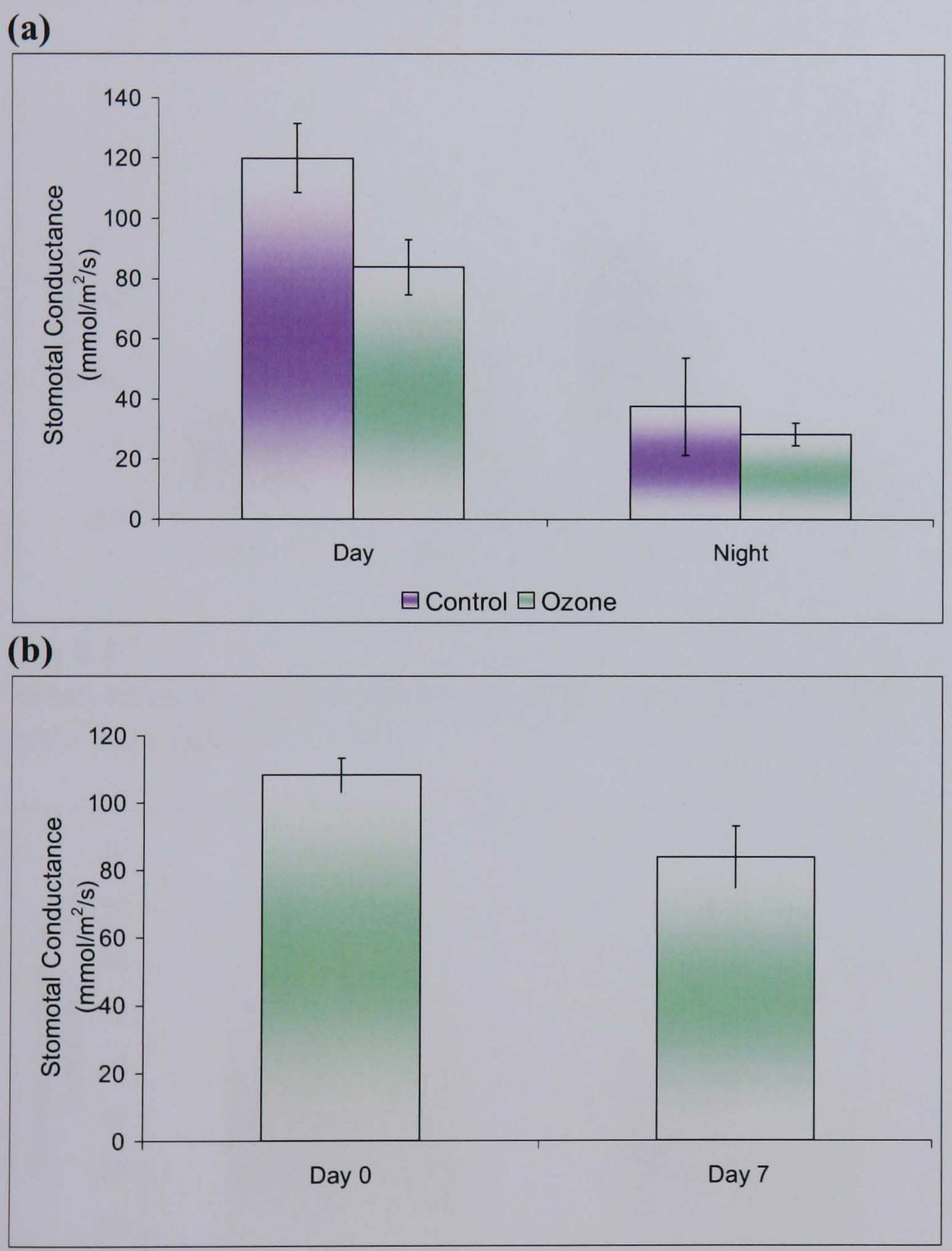


Fig 2.16
The mean stomatal conductance (mmol m^{-2} s^{-1}) of *Scrophularia nodosa* (a) at day 7 and (b) for ozone treatment at day 0 and day 7: error bars indicate +/- 1s.e.

Original in colour

2.3.8 *Valeriania officinalis*

Fig 2.17 gives the mean biomass values for *V.officinalis* per treatment. There was a 14% decrease in the root to shoot ratio for *V.officinalis* ($F = 3.76$; $P < 0.10$). However this does not correspond to any significant reductions in root or shoot biomass. There was a 29% reduction in stomatal conductance on day 19, at the end of the fumigation ($F = 3.33$; $P < 0.10$) (Fig 2.18).

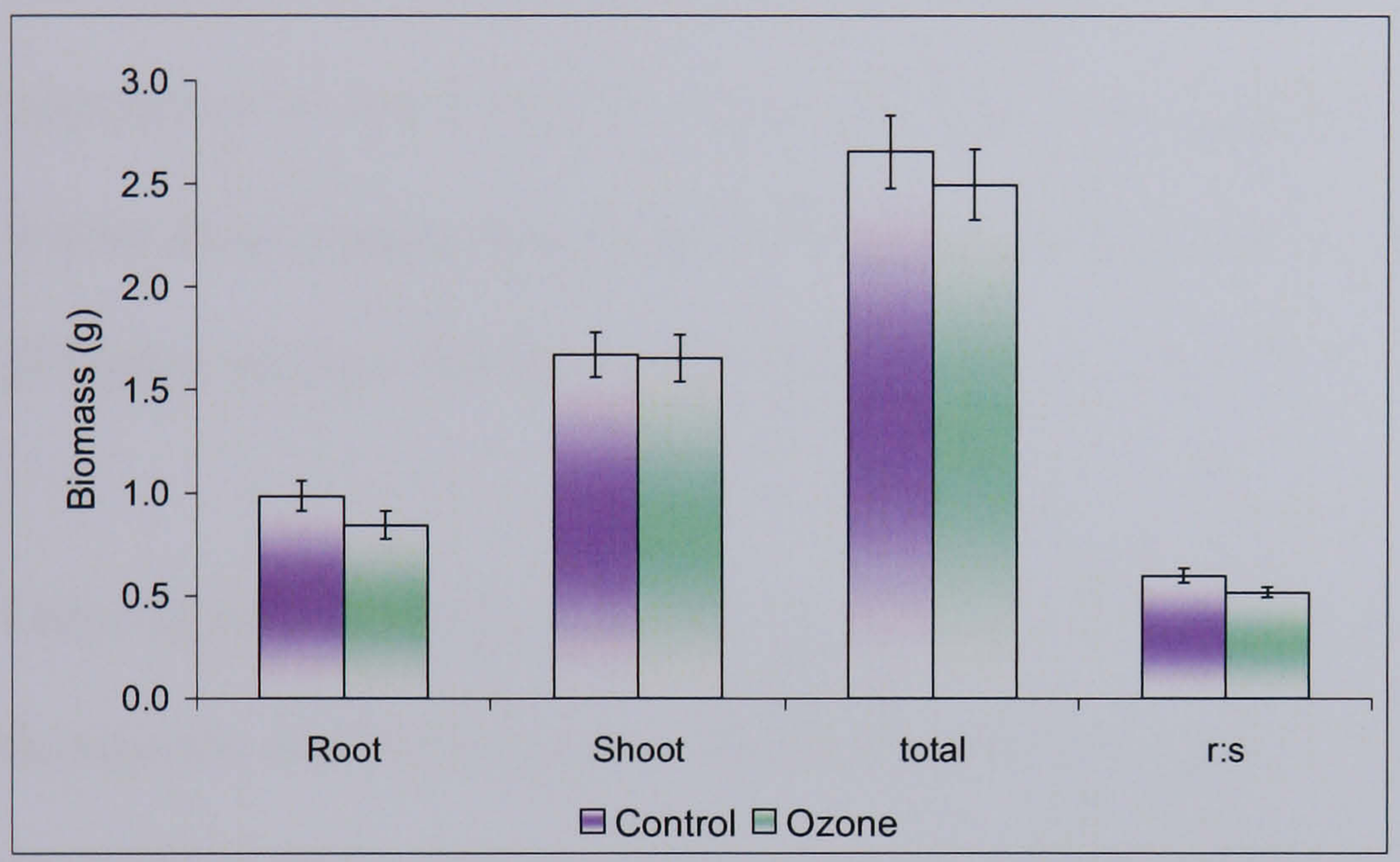


Fig 2.17
Mean total, shoot and root biomass (g), and root/shoot ratio (r/s) of *V.officinalis*: error bars indicate +/- 1s.e.

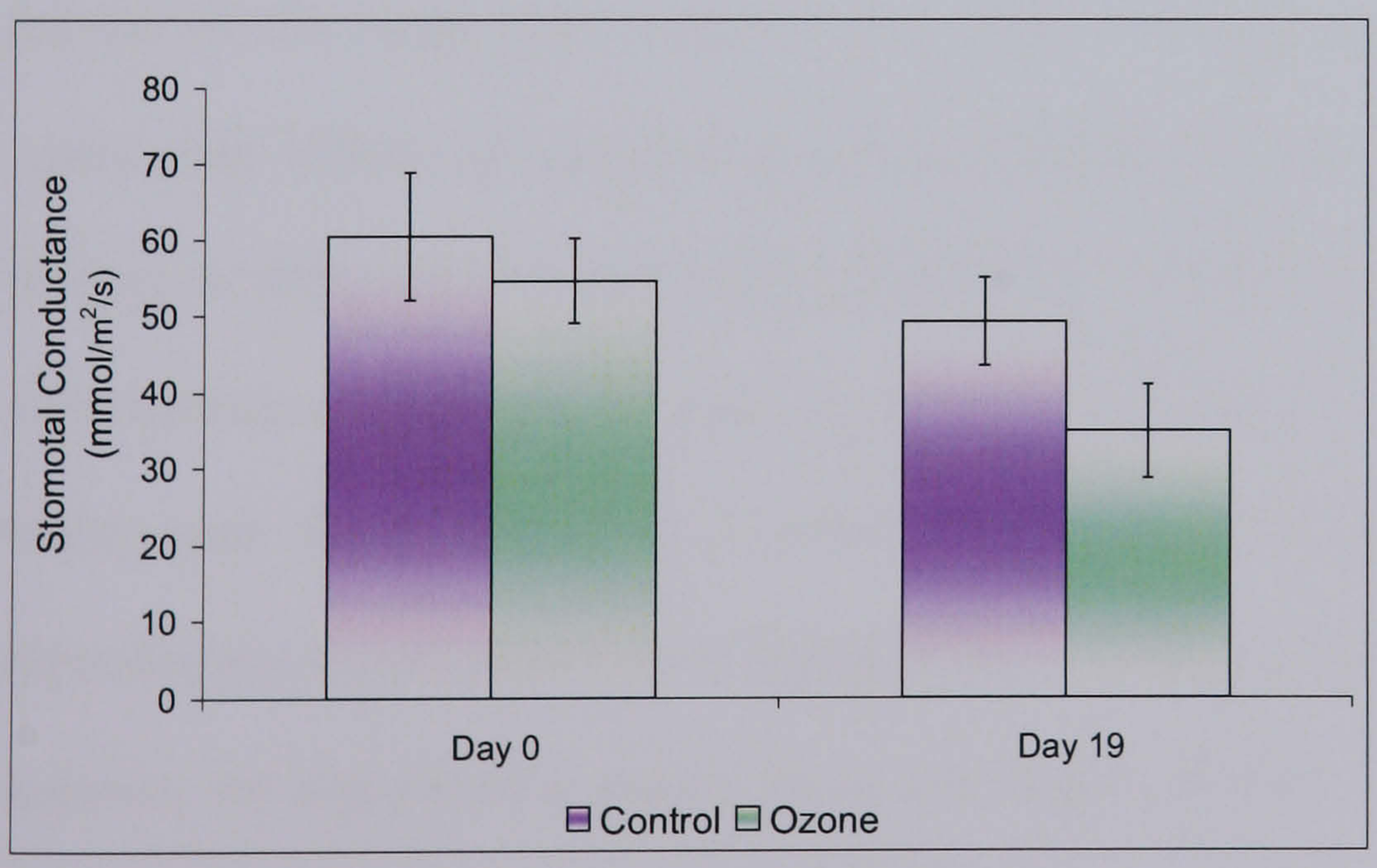


Fig 2.18
The mean stomatal conductance (mmol m^{-2} s^{-1}) for *V.officinalis*: error bars indicate +/- 1.s.e

2.4 Discussion

2.4.1 Limitations of study

The use of seed collected from the wild proved difficult, as many species collected did not germinate; those which did, often had small sample sizes, and this resulted in large variation caused through individual differences. However it was important to this series of experiments that the plants studied arose from the populations in the Yorkshire Dales and that they included the natural variation within these populations to get the best picture possible of the impacts of ozone pollution on these habitats and their ecotypes.

Other significant limitations arose mainly from two sources: (i) the short period of fumigation and (ii) the use of indoor exposure chambers. The small window of time used to assess the individual species does not allow maturation and reproductive output of the plants to be assessed. Further to this, a destructive harvest of the entire plant removes any scope for assessments of re-growth or 'carry over' effects of ozone on growth in subsequent seasons. There is evidence that ozone exposure can cause reduced reproductive output by reduced numbers of inflorescences, reduction in seed production and finally reduced germination ability and shoot vigour, in several crop species through direct effects on reproductive organs (Black *et al*, 2000). Effects of ozone, which may be direct or indirect, on reproductive output have also been reported in wild species (e.g. Bergmann 1996; Power & Ashmore, 2002)

However, the intention and design of the experiment was to use short- term fumigation studies to identify sensitive species and assess variation between habitats and populations. In this time, there were some large differences observed, both between and within species. The use of a relatively high concentration of ozone, 80ppb over the fumigation period means that the results have relevance to assessing the response of young plants to episodes of elevated concentrations of ozone. Concentrations of 80ppb are reached and exceeded during occasional episodes typically of 1-2 weeks duration during many British summers (NEGTAP, 2001).

Indoor exposure chambers are restricted in their ability to reproduce the outside environment. In particular lights provided only low photon flux densities of 90μmol m^{-2} s^{-1}, and consequentially the measured values of stomatal conductance were low. However these conditions may be more relevant for species growing as part of a woodland ground flora. For example, Rackham (1975) states that woodlands receive one third of full daylight in the spring in semi-natural coppice woodland. The low values of photon flux density, associated with low values of stomatal conductance, would lead to relatively low ozone flux into the leaves. However, they may also be associated with low rates of detoxification. This is especially apparent in shade species or in conditions similar to those in the forest understorey (Bjorkman 1981; Noguchi, Sonoike & Terashima 1996; Noguchi & Terashima 1997; Noguchi *et al.* 2005).

In a natural habitat, individual plants would be subjected to fluctuating levels of light, temperature and humidity over a period of time and within a 24 hour block.

Original in colour

However conditions within the chambers were stable and plants were exposed to ozone in unrealistic conditions. The experiment also excludes other sources of environmental variation such as competition, grazing and herbivory that would otherwise be present. Open-top chamber system or field fumigations would provide a more realistic alternative to indoor chambers. However, the lack of control of temperature and humidity in such facilities means that it is not possible to compare the response of species in different fumigation periods, as in this study.

2.4.2 Biomass responses

Effects on biomass were measured for all species, and thus provide a consistent basis for comparison of responses to ozone. Furthermore, this provides a basis for comparison with other studies of species responses to ozone, especially the work of Hayes *et al.* (2006a) who compared biomass responses of 83 species from a collation of experimental studies.

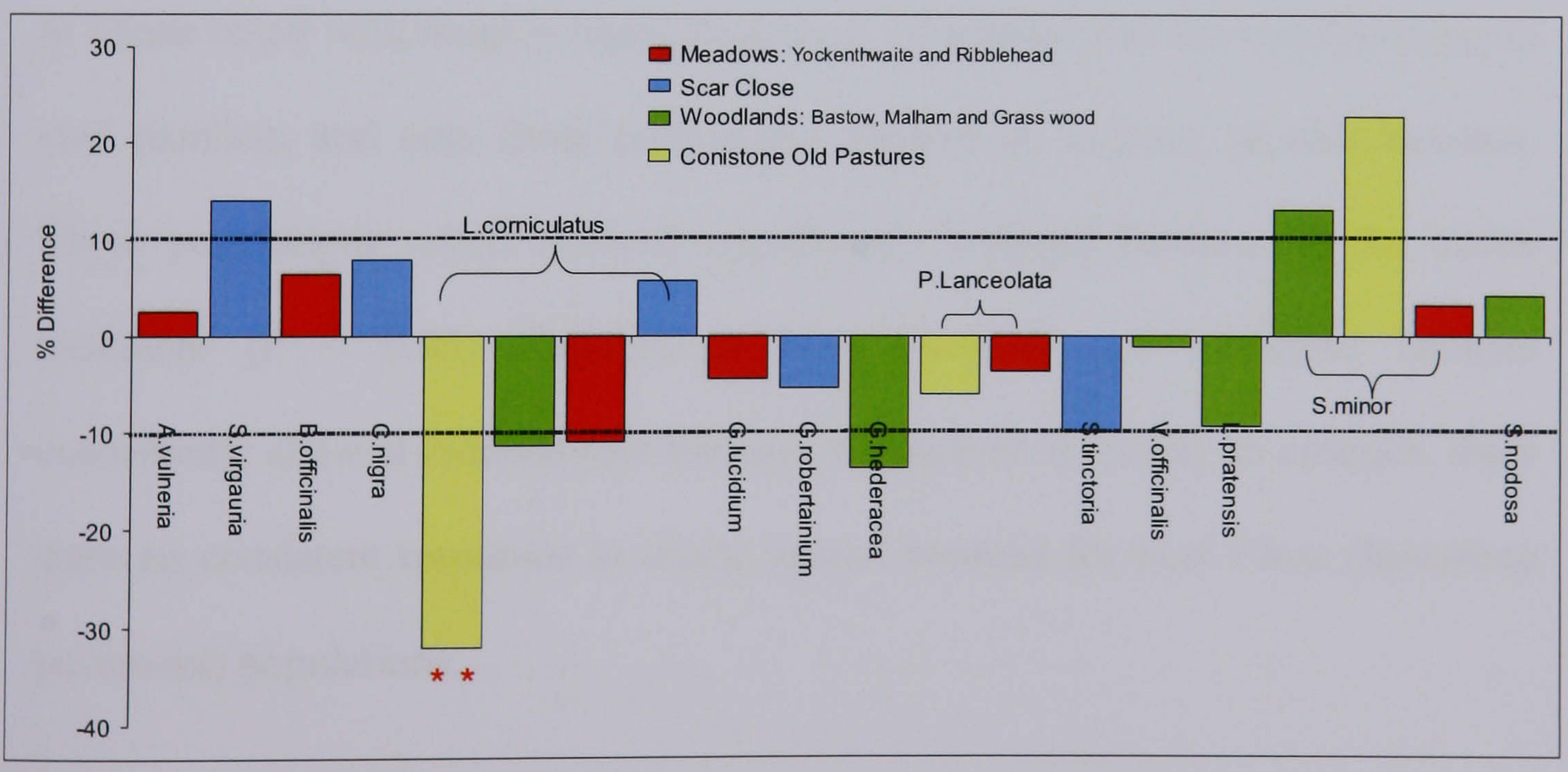


Fig 2.19
Summary of the responses in live dry above-ground biomass of 14 species to ozone fumigation (expressed as 100 x ((ozone-control)/control)).
Significant results are indicated as follows: * = *P<0.10;* ** = *P<0.05;* *** = *P<0.01*

Fig 2.19 summarises the percentage difference in mean above-ground biomass between the two treatments, indicating the different habitat types. Most differences between treatments are less than ± 10% and all but one is non-significant. There are no consistent differences between different habitat types; in each of the four habitat types, a similar number of species showed increases and decreases in above-ground biomass. A total of 9 species/populations showed an increase, and 11 species/populations showed a decrease, in above-ground biomass in response to ozone.

Fig 2.20 shows a summary of results for below-ground biomass, and provides a stark contrast to the results for above-ground biomass in Fig. 2.20. Many more species/populations show a negative response to ozone in terms of below-ground biomass than above-ground biomass. Eleven of seventeen tested populations showed decreases in below-ground biomass over 10% in response to ozone. In contrast to above-ground biomass, for which increases and decreases in response to ozone occur with roughly equal frequency, 14 populations showed decreases in root biomass, and only three populations showed an increase in root biomass. Three populations tested showed significantly reduced biomass in the ozone treatment ($P < 0.05$). Populations from meadow and woodland habitats consistently showed reduced root biomass in response to ozone. In contrast, there were no consistent responses to ozone in root biomass for Scar Close (limestone pavement) populations.

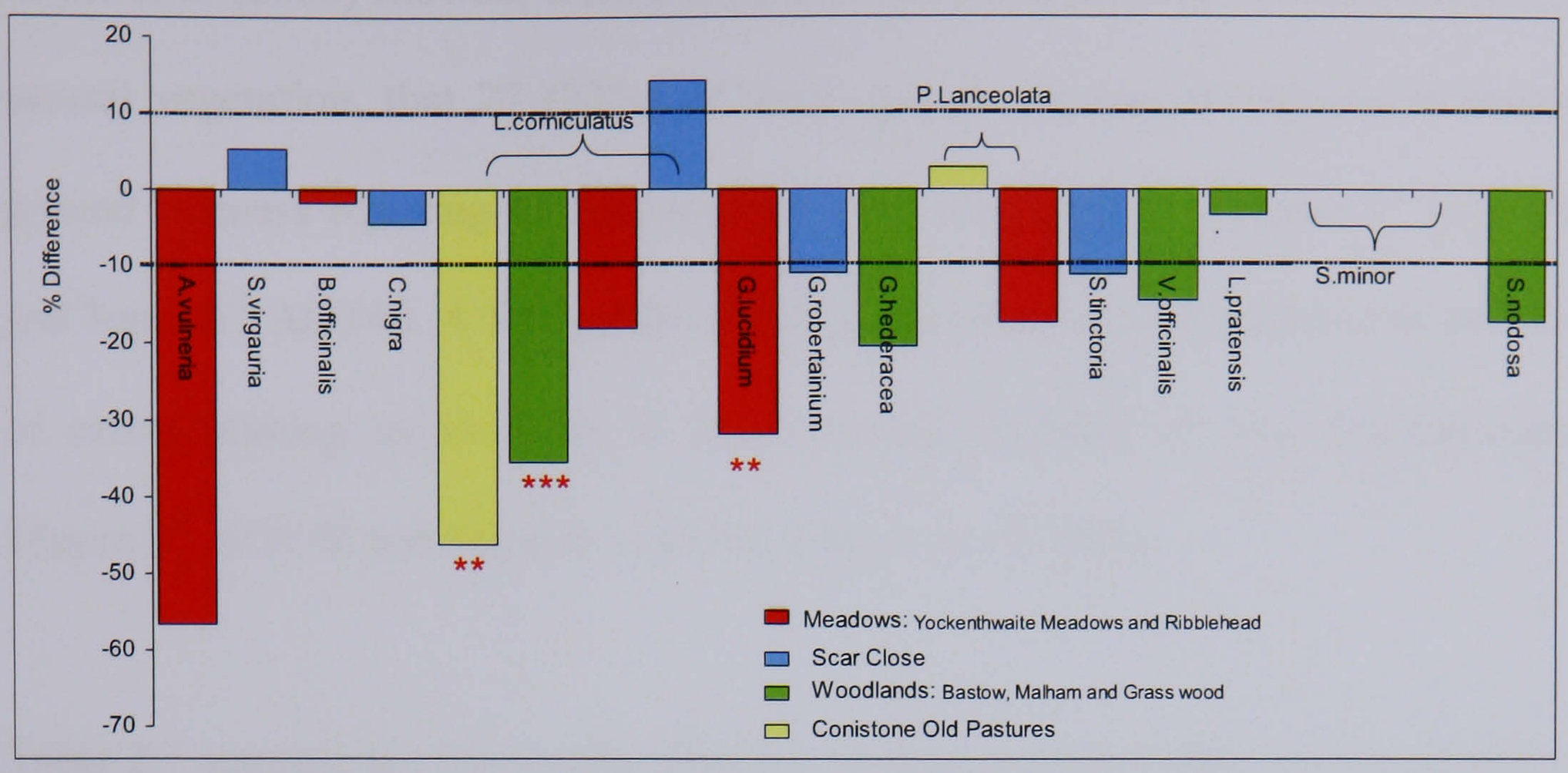


Fig 2.20
Summary responses in live root biomass of 14 species to ozone fumigation expressed as 100 x ((ozone-control)/control).
Significant results are indicated as follows:* = *P<0.10;* ** = *P<0.05;* *** = *P<0.01*

There are very few studies on semi-natural vegetation which have assessed ozone effects on root biomass. However, some studies have shown sensitivity in root biomass in response to ozone: Batty and Ashmore's (2002) study of wetland communities found that the roots of the species examined were far more sensitive to ozone than the above ground parts of the plant, particularly in *Digitalis pupurea* and *Epilobium hirstum*, which showed a 10% decrease in the relative growth rate of root biomass under low (AOT40 2000 ppb h^{-1}) ozone exposure. A study by Manninen *et al*. (2003) showed differences in ozone effects on roots between two populations of wild strawberries; one population showed a 13% decrease in roots where the other showed a 20% increase. Therefore there is not only evidence of ozone impacts on root biomass, but evidence that effects vary between populations of a species.

Hayes *et al* (2006) showed, from a review of 83 studies of ozone effects on semi-natural vegetation, that 27 (33%) of these species showed reductions in above ground biomass equating to decreases of over 10% between 15ppm h^{-1} AOT40 and 3ppm h^{-1} AOT40. A further 15 (18%) species showed a stimulation in growth of ozone relating to increases in above-ground biomass of over 5% between 15ppm h^{-1} AOT40 and 3ppm h^{-1} AOT40 (Hayes *et al*; 2006).

Table 2.7 summarises the results obtained by Hayes *et al*. (2006) for the Relative Sensitivity (RS) of the four species included both in their study and in this study. To provide a comparison with all species, relative sensitivity to ozone (RSp) was also predicted using the equation [RSp = 1.805-0.118Light-0.135√Salinity] developed by Jones *et al*, (2006). This was found to be an effective basis for estimation of the sensitivity of individual grassland species to ozone (Jones *et al*, 2006). The equation uses species specific Ellenberg indicator values for light and salinity. A value of RS or RSp of 1 indicates no change in biomass at 15ppm.h compared with 3ppm.h, while a value of RS or RSp of greater, or less than, 1 indicates an increase, or decrease, in above-ground biomass in response to ozone. For comparison, the biomass ratio (ozone/filtered air) for this experiment for above- and below-ground biomass was calculated.

The RS and RSp values (Table 2.7) indicated that most of the species included within this study would be only marginally impacted by ozone exposure (+/- less than 5% predicted change in biomass at AOT40 of 15ppm-h (Hayes *et al*, 2006)). The RS and RSp indices highlighted 6 species to be sensitive to ozone; these are *A.vulneria, G.robertanium, L.corniculatus, S.nodosa*, *S.virgerea* and *V.officinalis*.

Table 2.7
Ellenberg Indicator Index for Light and Salinity, Rs and Rsp values for all species in this study. RS_p = predicted relative sensitivity to ozone; RS = Relative sensitivity to ozone (Jones *et al*; 2006); - indicates no data available

Species	Ellenberg Index		RS_p	RS
	Light	Salinity		
Anthyllis vulneria	8	0	0.861	1.194
Centaurea nigra	7	0	0.979	-
Geranium lucidum	6	0	1.097	-
Geranium robertainium	5	0	1.215	-
Glechoma hederacea	6	0	1.097	-
Lathyrus pratensis	7	0	0.979	-
Lotus corniculatus	7	1	0.844	0.967
Plantago lanceolata	7	0	0.979	0.994
Sanguisorba minor	7	0	0.979	-
Scrophularia nodosa	5	0	1.215	-
Serratula tinctoria	7	0	0.979	-
Solidago virgireau	5	0	1.215	-
Stachys officinalis	-	-	-	-
Valeriania officinalis	6	0	1.097	0.805

A major conclusion is that, for above-ground biomass, the results of this experiment, the data of Hayes *et al*. (2006), and the model of Jones *et al*. (2006) all suggest that both small increases and decreases may occur in these species in response to ozone. In contrast, the results for roots in this experiment show a strong and consistent trend for decreases in root biomass in response to ozone. Roots are often neglected in studies of ozone sensitivity of semi-natural species and communities. From this investigation, the much greater negative effect on species in terms of root biomass than in above-ground biomass suggests that assessments of the relative sensitivity to ozone of species (Hayes *et al*., 2006_a) and communities (Mills *et al*., 2006) based only on above-ground biomass may be very misleading. There are many situations in which it could be argued that reduction in root biomass could have more significant long-term ecological implications for an individual plant than could losses in above ground biomass.

The roots are certainly at risk of predation from root feeding insects and molluscs. They also serve as the nutrient and water uptake point for a plant. Therefore loss of root biomass will likely have large consequences for slow growing, perennial species which rely on roots for regeneration in the subsequent seasons, while decreases in root biomass may lead to reduced uptake of water and nutrients. For example, woodland species often seek out nutrient rich patches, as this type of habitat is typically nutrient poor (Wijesinghe *et al.*, 2001). The changes in carbon partitioning in favour of shoots over roots in this experiment could directly relate to nutrient deficiency indicated by chlorosis and increased senescence.

2.4.3 Differences between populations

2.4.3.1 Plantago lanceolata

There were no significant differences in biomass between populations for *P.lanceolata* and there were no significant differences in the different populations' response to ozone treatment. However, both populations showed a significant reduction in leaf width under ozone.

P.lanceolata is known to have large genetic variability relating to huge amounts of phenotypic plasticity (Kuiper and Bos, 1992; Van Tienderen, 1990; Antonovics & Primack, 1982 & 1981). Fig 2.21 gives the relative frequencies of distribution, between populations and treatments, of two phenotypes of *P.lanceolata*. These are the 'prostrate' form with leaves lying horizontal and the 'upright' form with vertical leaves. At the end of the experiment individual plants were classified as one or the other. Upright phenotypes are typically associated with hayfield environments such as Yockenthwaite Meadows and prostrate forms are typically

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associated with pasture and grazed environments such as Conistone Old Pastures (COP) (Van Tienderen, 1990).

Both populations showed a decrease in frequency of the prostrate phenotype in ozone (Fig 2.21). There was no significant treatment effect on the proportion of the two phenotypes for the Yockenthwaite Meadows population, but the Conistone Old Pastures population has a significant treatment effect on the proportion of phenotypes ($\chi 2 = 3.09$; $P < 0.10$). In the control treatments, the two phenotypes are present in roughly equal proportions, whereas in the ozone treatments the upright form is present in much higher proportions. It could be that, due to the phenotypic plasticity and adaptability of *P.lanceolata* to current environmental conditions, the upright form is more advantageous in high concentrations of ozone for this population.

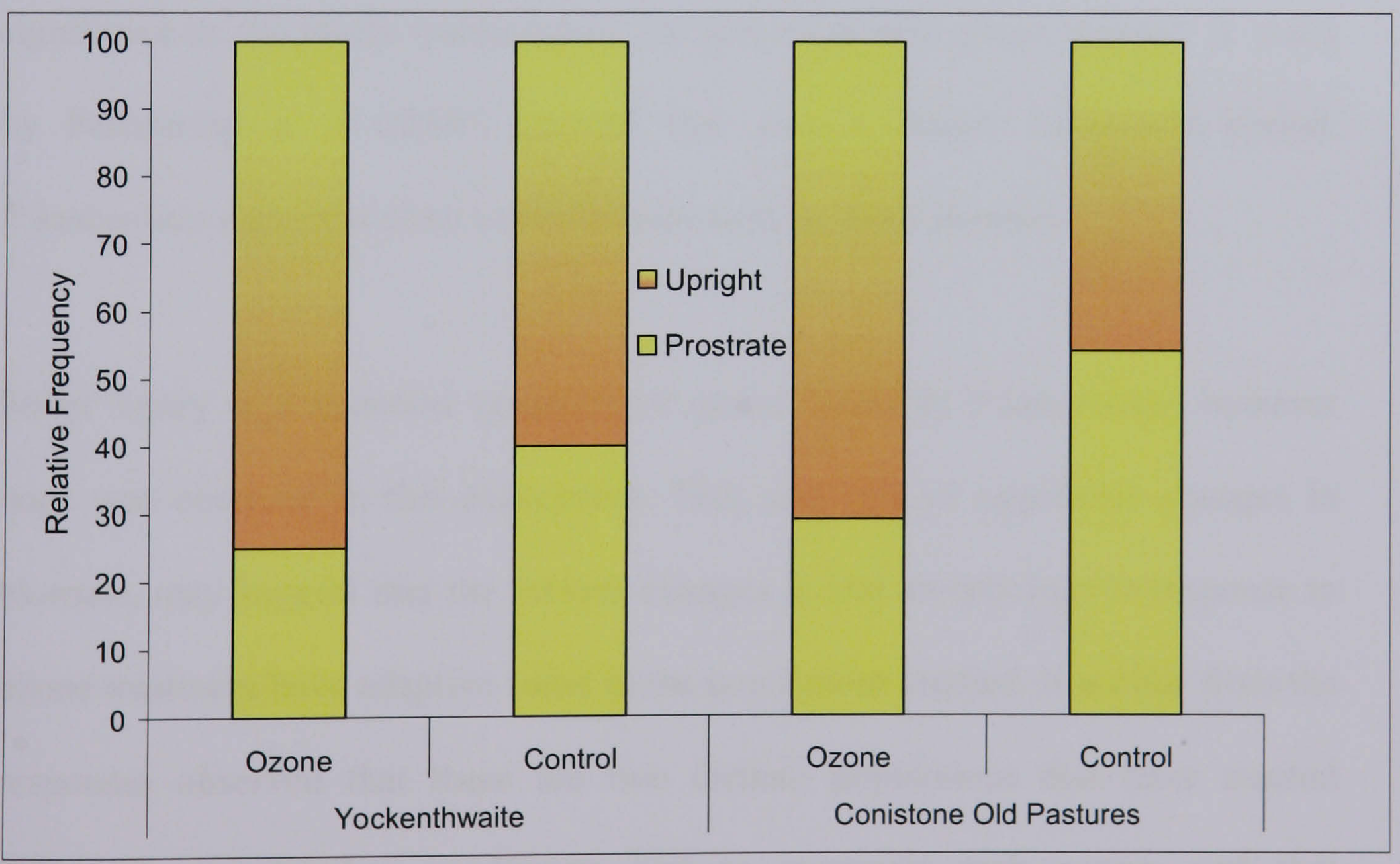


Fig 2.21
Relative frequency of two Phenotypes of *P.lanceolata*

Both populations also displayed narrower leaves within the ozone treatment; this could be a phenotypic adaptation to ozone conditions rather than a negative response, reducing water loss from damaged tissue or reducing the surface area available to gas exchange.

Investigations into carbon partitioning in *P.lanceolata* showed that hayfield and meadow community populations, such as the Yockenthwaite population described here, have a higher tendency to invest in shoot growth over root growth (Van Tienderen, 1990). The Yockenthwaite population also had a significantly greater leaf area than the Conistone population ($F = 52.93$; $P < 0.01$). It is interesting that this population have small (not-significant) reductions in above-ground biomass but large reductions (although non-significant) in below-ground biomass. If the experiment have proceeded longer would the root reductions have become more significant as the plants compensated for loss of growth above ground? A study by Franzaring *et al* (2000) showed that, over a longer fumigation period, *P.lanceolata* showed a trend towards reductions in shoot biomass.

Foliar injury is a common symptom of ozone injury in *P.lanceolata*, however none was observed in this experiment. This, and lack of significant changes in biomass, may suggest that the various changes in leaf morphology in response to ozone treatment have adaptive value in the populations studied. It is clear from the responses observed that these are two distinct populations that have reacted differently to chamber conditions, and to treatment with ozone, and that differences in their morphology relate to their habitat type.

2.4.3.2 *Lotus corniculatus*

Physical differences between populations of *L.corniculatus* were more easily distinguishable than for *P. lanceolata*. Populations from Scar Close showed a non-significant positive stimulus from ozone treatment whereas all the other populations showed decreases in below-ground and above ground biomass. The Conistone Old Pastures was the smallest of the four populations and showed the largest reductions in biomass above and below ground. This population also had the fewest leaves, demonstrating overall that is was physically smaller than the other three. However there was no significant population or population x treatment effects, most likely due to the high amounts of within population variation.

2.4.4 Individual Species Responses

2.4.4.1 *Geranium lucidium*

G.lucidium showed a significant increase, in the ozone treatment, in the reddening of the leaves. This was most likely related to increased levels of anthocyanin; Anthocyanins were identified in the leaves of *Rumex crispus* following exposure to smog; these had caused the leaves to flush with red pigmentation (Koukol & Dugger, 1967).

Anthocyanins are known antioxidants and there is evidence to suggest that they may be formed to reduce photo-oxidative damage (Bussotti *et al.*, 2007). However in this study *G.lucidum* leaves in the ozone treatment were also smaller with a significantly lower mean individual leaf area suggesting that there was some negative response to ozone exposure. This suggests that the presence of

anthocyanins is not fully compensating exposure to ozone for this species, or is not acting to remediate damage.

Other studies have seen a similar response in different species when exposed to ozone. Reddening of leaves attributed to the increased levels of anthocyanins in ozone exposed plants has been reported in *Centaurea jacea, Knautia arvensis, Lychnis flos-cuculis*, (Bungener *et al*., 1999) and *Rumex obtusifolius, Scenecio vulgaris and Sonchus asper* (Bergmann *et al*., 1995). Increased levels of anthocyanin have been reported in *Calluna vulgaris* which was found to have 43% increases in anthocyanin content following a frosting and an ozone episode (Foot *et al*, 1996). In an exposure study on two different populations of wild strawberries, in which there was increased reddening of leaves with age, one population showed significant increased reddening of the leaves when exposed to ozone (Manninen *et al*; 2003). This also suggests that differences may exist between populations and provides scope for further study.

2.4.4.2 Valeriana officinalis

V.officinalis is typically a plant common of wetland areas, in this study the populations were found in a woodland valley in slightly marshy conditions, where the soils were moist. It has been shown that wetland plants are vulnerable to ozone pollution (Power & Ashmore, 2002); in a series of open-top chamber experiments, *V.officinalis* was found to be particularly sensitive to ozone in terms of biomass, showing large decreases in both root biomass and above ground biomass. In Power & Ashmore's (2002) study, commercial seeds were used. Natural wild populations are likely to be far more genetically variable with large differences between individuals. However, the wild V.*officinalis* population used

in this experiment showed a significant 14% reduction in root to shoot ratio. In contrast to the losses below ground, the leaves were healthy with no signs of visible injury, but did have significantly reduced stomatal conductance, an effect also reported for this species by Power & Ashmore (2002). Reduced conductance would reduce the flux of ozone and therefore cause less damage, but also reduce the photosynthetic rate, perhaps causing the observed change in root/shoot partitioning.

2.4.4.3 Scrophularia nodosa

S.nodosa is a shade-tolerant (Ellenberg Light = 5) woodland plant, and hence in the chambers it was under typical low light conditions. The calculated RSp values for *S.nodosa* (Table 2.3) is 1.215 suggesting that ozone will have a stimulatory effect on growth. However, in this study, *S.nodosa* showed a reduction in stomatal conductance under ozone exposure and had significant reductions of root biomass (17%). Foliar injury was also quite extensive to the leaves, but there was no significant effect on the biomass of the above ground parts of the plant, even with the large amounts of visible injury. Hence the RSp value based on the model of Jones *et al*. (2006) seems inappropriate for this population.

2.4.4.4 Glechoma hederacea

G.hederacea is also a common species of woodlands and is a semi-shade species (Ellenberg Light = 6). The calculated RSp value (Table 2.3) is 1.097 but this predicted value seems inappropriate as this species also showed large effects of exposure of ozone. *G.hederacea* exhibited changes in leaf morphology in the ozone treatment, having many small leaves in contrast to the control which had fewer larger leaves, and also showed large amounts of foliar injury. Within a

woodland environment, where competition for light is the priority this species may lose out if ozone exposure leads to smaller damaged leaves.

2.4.5 Habitat differences

The most sensitive species, based on the below-ground responses that were the largest and most consistent effects of ozone, came from meadow and woodland communities. It is clear from the results that none of the species or populations from Scar Close was susceptible to ozone. However, growth of the species from this habitat may have been a factor. Three of these species are from the family the Asteraceae; *S.tinctoria, S.virgireau* and *C.nigra*. All three species were difficult to grow, and this resulted in fairly small sample sizes for *C.nigra* and *S.virgireau* and some very small sickly looking plants for *S.tinctoria.*

Woodland species consistently showed significant responses to ozone in terms of biomass and other indices. Many of these woodland species showed signs of foliar injury, even where there were no other measurable ozone effects. This may be indicative of the potential for longer-term effects of ozone on these species, relating to growth outside of the fumigation period, over-wintering ability and fecundity. This study thus suggests that species from woodlands may be particularly sensitive to ozone. The reasons for such sensitivity could be related to the marginal nature of many woodland herb species and their various adaptations to life within the woodland canopy. Hayes et a. (2006) reported that species with a lower Ellenberg shade index (i.e. those adapted to lower light) were less sensitive to ozone; however, their database only included species with an Ellenberg light index from 5 to 9, and hence did not include specialist woodland shade species.

There are, however, very few studies that focus on the effects of ozone on individual species of woodland ground flora. Barbo *et al* (1998) examined the growth of an early successional woodland plant community in open top chambers; they found that ozone exposure caused shifts in competition, resulting in reduced biodiversity and changes in community composition. Hence, there may be a potential for the effects on individual species shown in this experiment to lead to changes in competitive balance in woodland ground flora of upland UK woodlands of high conservation value. However, the short term nature of this study and the laboratory conditions prevent extrapolation to reproductive output and actual fitness in a natural environment.

3. Chapter 3: The Effects of Ozone Exposure on Native Populations of Spring Flowering Bulbs.

3.1 Introduction

British woodlands are renowned for their showy displays of woodland flora in the spring. These often unbroken carpets are classed as a national treasure. The importance of woodlands as conservation areas for wildlife and for recreation and enjoyment should not be overlooked. However, woodland habitat in the UK is declining in area, is being cleared for construction and much is in a degraded state (Spencer & Kirby, 1992). Upland woodlands incorporate areas of rare habitats; two are identified in the UK Biodiversity Action Plan (BAP), these are: limestone woodlands, particularly *Fraxinus excelsior - Acer campestre - Mercurialis perennis* woodland, and upland broadleaved oak woodland. Woodlands provide important habitat not just for the plant species which comprise it, but for many invertebrates, birds and mammals. Much of the woodland in an area like the Yorkshire Dales National Park is fragmented and impacted by the effects of grazers.

Kirby at al (2005) found a 12% decrease in species richness at woodland sites over a 30 year period. They suggested that the major drivers of change in British woodlands over the last 30 years are those deriving from increasing pH, from eutrophication via nitrogen deposition, and from lack of management resulting in increasing shade within some woodland (Kirby *et al*; 2005).

The deciduous nature of much native British woodland is such that in the spring period the forest floor is exposed and uncovered by the forest canopy. This allows for the spring flowering patterns of bulb species such as *Hyacinthoides non-scripta* and *Allium ursinium*, which grow, flower and reproduce before the forest leaf canopy has fully matured.

Ozone levels are set to increase in upland habitats in the future especially in the spring periods when northern hemispheric background ozone concentrations are at their highest (Coyle *et al.*, 2003); this may lead to an important shift of ozone exposure towards earlier spring periods in the future. However, with rising background levels of ozone, are the species of woodland ground flora sensitive to this pollutant? Despite the considerable number of studies of ozone effects on wild species, there has been no published study on the important group of spring bulb species.

This study aimed to identify species sensitive to exposure to ozone among the spring flowering bulb species of British woodlands. In particular this study concentrated on the sensitivity of populations of species from upland woodland within the Yorkshire Dales National Park at Grass Wood SSSI, North Yorkshire. This was the woodland site from which the soils taken for the mesocosm experiment described in Chapter 5 were collected, and in which the field measurements described in Chapter 4 were made.

Woodland perennial species such as *H.non-scripta* and *A.ursinium* regenerate seasonally from a bulb. For *H.non-scripta,* although seed is set every year,

establishment from seed is infrequent and seeds do not persist in the seed bank (Warr *et al.*, 1994), a mature plant can produce 2-4 daughter bulbs per annum (Blackman and Rutter, 1954). With most new recruitment to populations via vegetative reproduction and population sustainability relying on vegetative expansion, any negative effects of ambient ozone on carbon partitioning between above and below ground parts of the plant could have serious implications for such species. Hayes *et al* (2006a) demonstrated significant carry-over effects to the following year's growth of grassland species caused by ozone exposure. Therefore, this study focussed in particular on the relationship between shoot growth (annual growth) and bulb growth (perennial storage).

3.2 Methods

Three species of spring flowering bulbs were collected, two native species of Grass Wood, *Allium ursinium* and *Hyacinthoides non-scripta*, and one from a commercial source: *Narcissus pseudonarcissus*. These were then exposed to ozone in an indoor exposure chamber system (described in detail in Chapter 2) over a 24 week period.

3.2.1 Bulb collection and planting

Allium ursinium and *Hyacinthoides non-scripta* bulbs were taken from wild populations in Grass Wood. Bulbs were collected from Grass Wood in December 2004 with a quantity of soil from the site of collection. The *H. non-scripta* bulbs were taken from the mature woodland area described in Chapter 5, the *A.ursinium* bulbs were taken from a stand of *A.ursinium* plants near the northern edge of the wood. Soil was mixed 70:30 with commercially produced bulb mulch and the bulbs were potted into 9cm x 9cm x 10cm size pots. Prior to planting, the bulbs were washed with deionised water to remove any residual soil and then weighed to give an estimate of the initial bulb fresh weight. Bulbs were distributed between chambers depending on this initial bulb weight, so each of eight chambers had bulbs of equal mean initial weight.

Narcissus pseudonarcissus bulbs were obtained from a commercial source (B&Q) and planted into 13cm x 13cm x 13cm pots filled with commercial bulb mulch. *N.psuedonarcissus* was not present in Grass Wood and was included to increase the number of spring flowering bulbs in the study; it is however common in other upland areas. *N.psuedonarcissus* bulbs were of a uniform size and therefore were

not weighed before planting. Pots were then immediately placed into an exposure chamber on 12th December 2004.

3.2.2 Experimental Design

Bulbs were then distributed into eight chambers; 4 were supplied with charcoal filtered air (CFA) and 4 were supplied with a target concentration of 80ppb (AOT40 360 ppb d^{-1}) ozone for 9 hours a day. The pots were placed in the chambers on 13th December 2004 and fumigation ended on 2nd May 2005, giving a total of 24 weeks, with 143 days of fumigation (7 days off over Christmas 2004). Details of the exposure chamber system are given in Chapter 2 (Table 2.2); however, in this experiment, the chambers were kept at 22°C during the day and 15 °C at night and humidity levels were kept at 80%. The pots were maintained in a moist soil with regular watering. The following numbers of plants were placed in each chamber: *A. ursinium*: 6; *H. non-scripta:* 5; *N.psuedonarcissus*: 5. Plants were monitored regularly for any signs of visible foliar injury. No symptoms were observed for *H. non-scripta* and *A. ursinium*.

N. pseudonarcissus bulbs became infected by an unknown fungal disease, causing a white covering to the bulbs and soil; however it was decided not to treat these plants with a fungicide. These plants did grow to maturity, but by the conclusion of the fumigation period, there were signs of chlorosis on some leaves and the shapes of the leaves were irregular, wavy and many lay horizontal rather than upright. These symptoms were noted and given a rank in order of their severity; these ranks are given in Table 3.1.

Table 3.1
Description of foliar abnormality and rank of symptoms of *N.psuedonarcissus*

Rank	Leaf Chlorosis	Leaf Deformity
0	No damage all leaves green and healthy	All leaved upright and turgid
1	Small scale damage >5% to all leaf surfaces	1-2 leaves distorted in shape and or lying horizontal
2	Larger scale damage >50% of leaves	50% of leaves distorted, horizontal and loss of turgidity
3	Chlorosis present on all leaves	No leaves in a healthy upright, turgid position.

Following the 24 week fumigation period, all individual plants were separated into their component parts and dried for 48 hours in an oven. The number of flowering heads was counted at the end of the experiment only, as there was little flowering. These were then weighed and recorded. Table 3.2 gives details of biomass categories for each species.

Table 3.2
Plant component part division for biomass

	H.non-scripta	*A.ursinium*	*N.psuedonarcissus*
Root	none	Root only	Root only
Bulb	Root and Bulb (root small and inseparable)	Bulb only	Bulb only
Leaves	Leaves	Leaves	Leaves
Flowers	None	Flower heads and flower stalks and any buds	Flower heads and flower stalks and any buds
Total Biomass	All the above	All the above	All the above

3.2.3 Statistical analysis

The data for all individuals were collated into two treatment groups and tested for the assumptions of ANOVA using SPSS (version 14, SPSS inc) and were then tested using a two-way analysis of variance (block vs. treatment). This method ensured there were no significant chamber effects and as no significant block interactions occurred, there is confidence that there were no chamber effects. When initial bulb weights were measured they were used as a covariate in the analysis of variance. Due to the variable nature of the material used significance was accepted at $P < 0.10$.

3.3 Results

3.3.1 Introduction

Table 3.3 gives a summary of the ANOVA results for effects on biomass between treatments. For *A.ursinum* and *H. non-scripta* the initial bulb weights were recorded and used as a covariate in the analysis.

Table 3.3
Summary results table for ANOVA (1.d.f.) for between treatment effects without initial bulb weight as a covariate and with the use of the covariate.
* $p < 0.01$; ** $p < 0.05$

	F-values		
	Allium ursinium	*Hyacinthoides non-scripta*	*Narcissus pseudonarcissus*
Initial bulb weight	0.00	2.32	-
Without covariate			
Number of leaves	0.67	1.84	
Bulb biomass	2.74	2.99*	0.81
Root biomass	1.96	-	1.76
Leaf biomass	2.29	2.60	0.99
Flower biomass	0.02	-	0.31
Dead biomass	-	-	0.18
Total biomass	3.00	3.18*	1.19
With covariate			
Number of leaves	0.70	1.21	-
Bulb biomass	4.17*	0.70	-
Root biomass	3.55*	-	-
Leaf biomass	2.60	1.21	-
Flower biomass	0.02	-	-
Total biomass	5.81**	0.89	-

3.3.2 *Hyacinthoides non-scripta*

Initial wet biomass of *H. non-scripta* bulbs after their distribution between treatments was not significantly different; Fig 3.1 displays the average biomass of *H. non-scripta* at the start of the experiment.

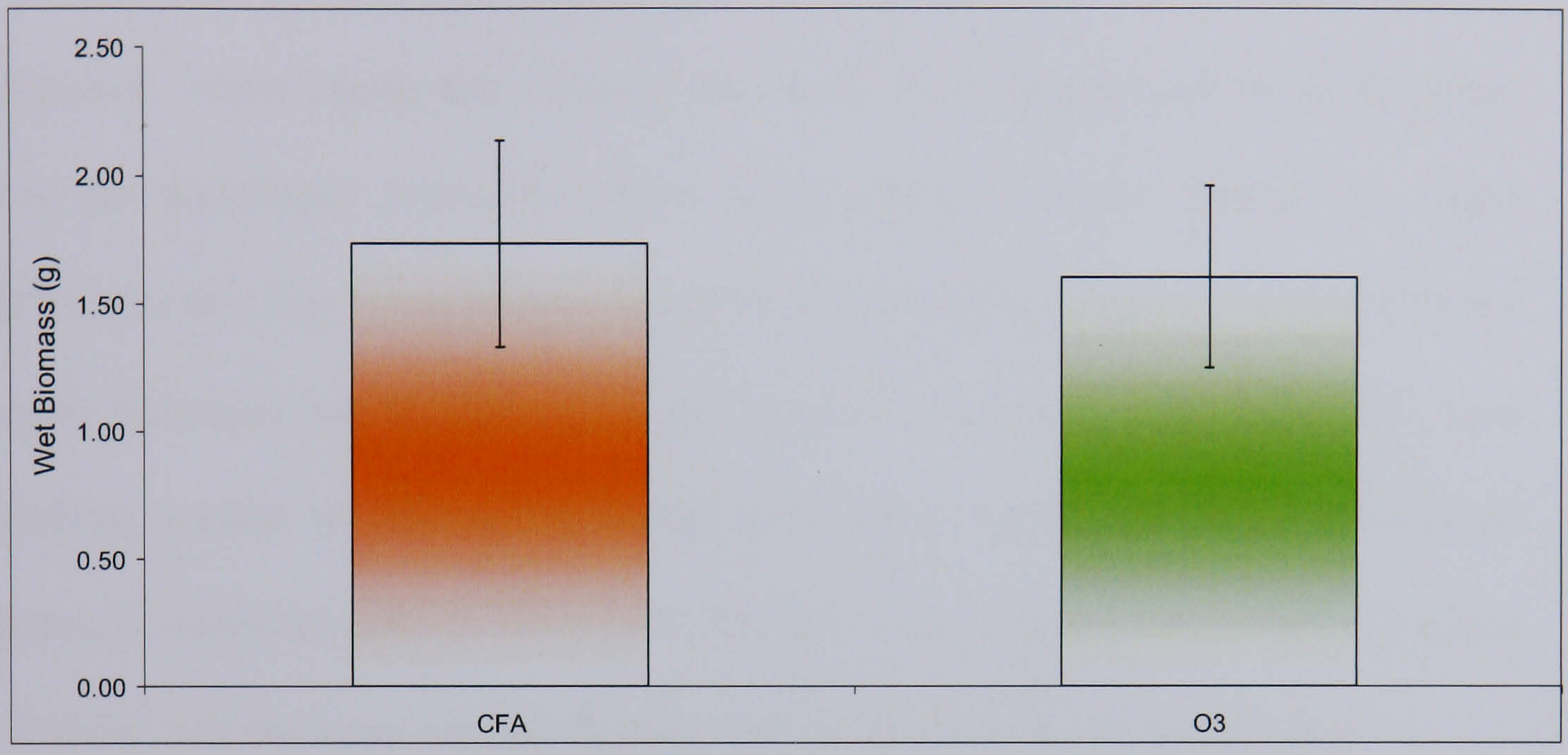


Fig 3.1
Initial wet biomass of *Hyacinthoides non-scripta* bulbs prior to ozone exposure. Error bars represent +/- 1 s.e.

Fig 3.2 shows the mean biomass values for the bulb and leaf components of *H.non-scripta*, and the total biomass, at the end of the exposure study. Biomass of all individuals was low; over the 24 week period there was very little growth in both treatments and no individuals reached a flowering stage.

However bulb biomass was lower in the ozone treatment than the control, with a reduction of approximately 41% ($F = 2.99$; $P < 0.10$), and in total biomass, with a reduction of 43% ($F = 3.18$; $P < 0.10$). At the end of the experiment mean (± 1s.e.) bulb dry-weight in the CFA treatment was: 0.29 ± 0.053 g; and in the ozone treatment was: 0.17 ± 0.032 g ($t = 1.91$; $p < 0.10$; d.f. = 24). This was because six bulbs did not grow from the control treatment, with an average initial weight of

0.711 ± 0.175g, while seven did not grow from the ozone treatment, with an average initial weight of 1.987 ± 5.994 g. In general the bulbs that did not grow were small, but some large bulbs did not grow in the ozone treatment, whereas in the control treatment all large bulbs grew well.

However, when taking into account the initial fresh weights of the bulbs, there was no significant treatment effect on dry bulb biomass. Hence, the main difference that has occurred was due to the lower initial weight of the bulbs in the ozone treatment due to failure of many to grow; excluding un-grown bulbs from analysis results in almost significant differences in initial wet bulb weight between treatments ($t = 1.711$; $p = 0.10$; d.f. = 24). Futhermore, when including all bulbs, wet biomass was not significantly different between treatments.

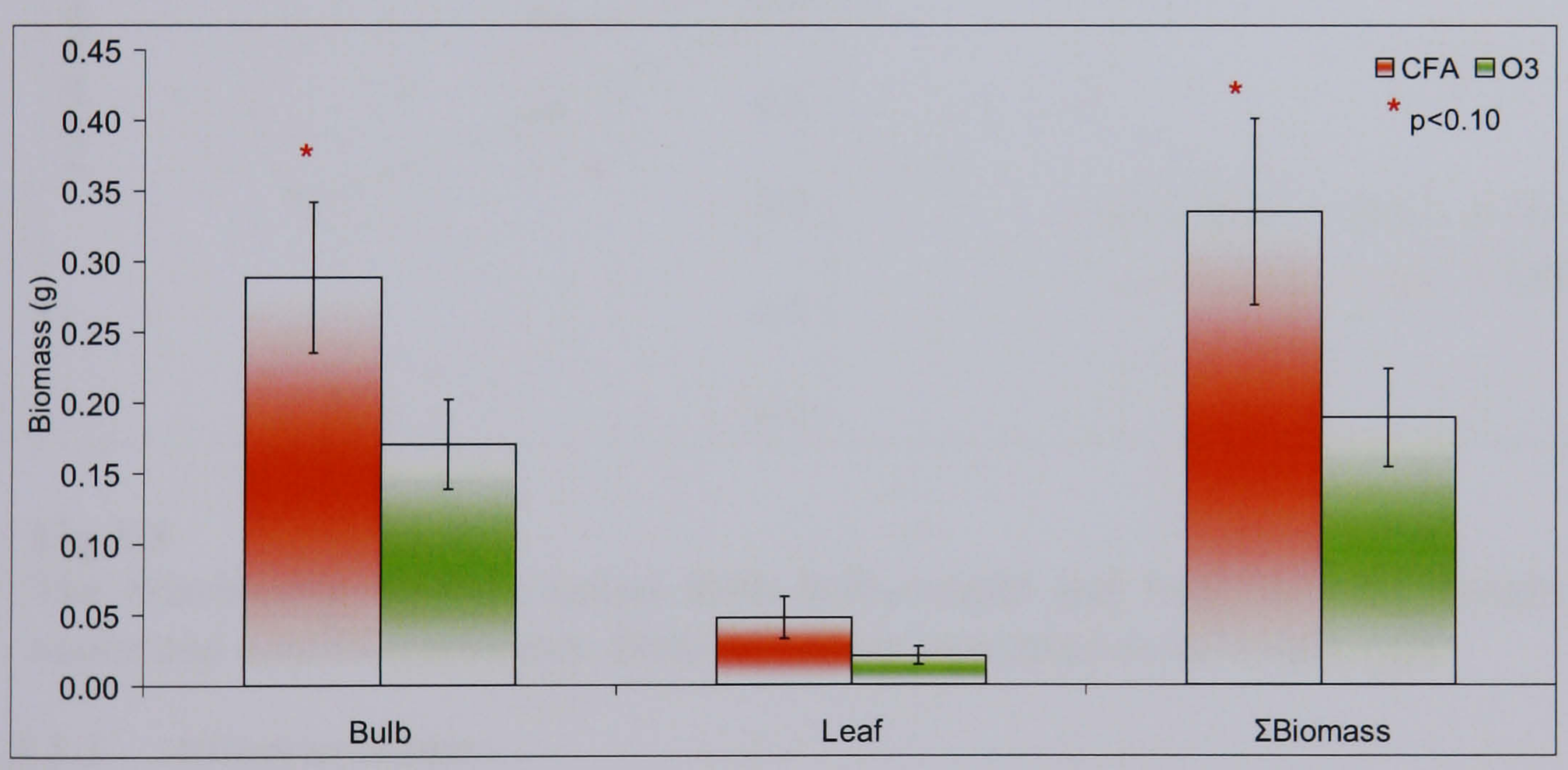


Fig 3.2
Biomass of *Hyacinthoides non-scripta* at the end of the experiment.
Error bars represent +/- 1 s.e.

Fig 3.3 shows the relationship between the bulb weights at the start and at the end of the experiment, for those individuals that survived to the end of the fumigation period, expressed on a log basis. There is a good fit to the data in both treatments (Pearson's correlation coefficient (r) = 0.712; $p < 0.01$ in control and $r = 0.850$; p

< 0.01 in ozone treatments). The slope of the fitted line is similar in ozone and control treatments; although the intercept is significantly lower in the ozone treatment. This strongly suggests that final bulb weight is largely dependent on the initial bulb weight. However, while the initial bulb weight was generally lower in the control treatment, in the range where initial bulb mass was comparable (between 0.5 and 1.5 g), the bulbs in the ozone treatment had a consistently lower final dry weight.

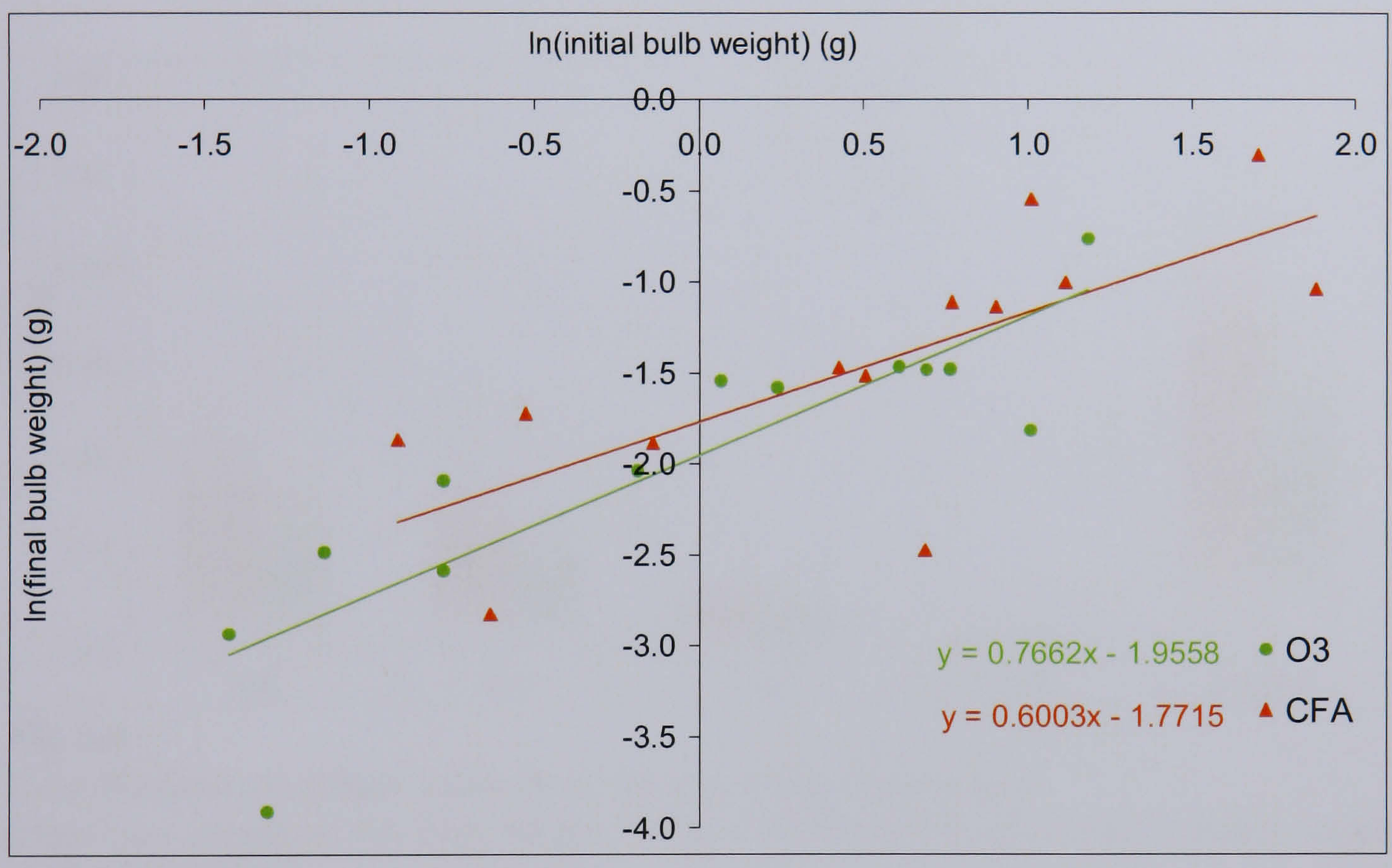


Fig 3.3
The relationship between initial fresh bulb weight and final bulb dry weight, in ozone and control treatments. Both values are expressed as ln(weight (g)).

3.3.3 *Allium ursinium*

Fig 3.4 shows the final mean live biomass for the component plant parts and the total live biomass for *A.ursinium* following a 24 week fumigation period. These plants grew more successfully than the *H. non-scripta* plants, with three plants reaching a flowering stage, and at the time of harvest the plants were just starting to die back.

There was no significant difference between treatments in initial wet bulb weight (Table 3.3). There was also no significant difference between treatments in bulb dry weight at the termination of the study. However, there was a significant reduction of 28% in bulb biomass and root biomass in the ozone treatment using initial fresh bulb biomass as a covariate this is significant (bulb: $F = 4.17$; $P < 0.05$; root: $F = 3.55$; $P < 0.10$). Adjusting for initial bulb weight, total biomass ($F = 5.81$; $P < 0.05$) was also significantly reduced in the ozone treatments by 27%.

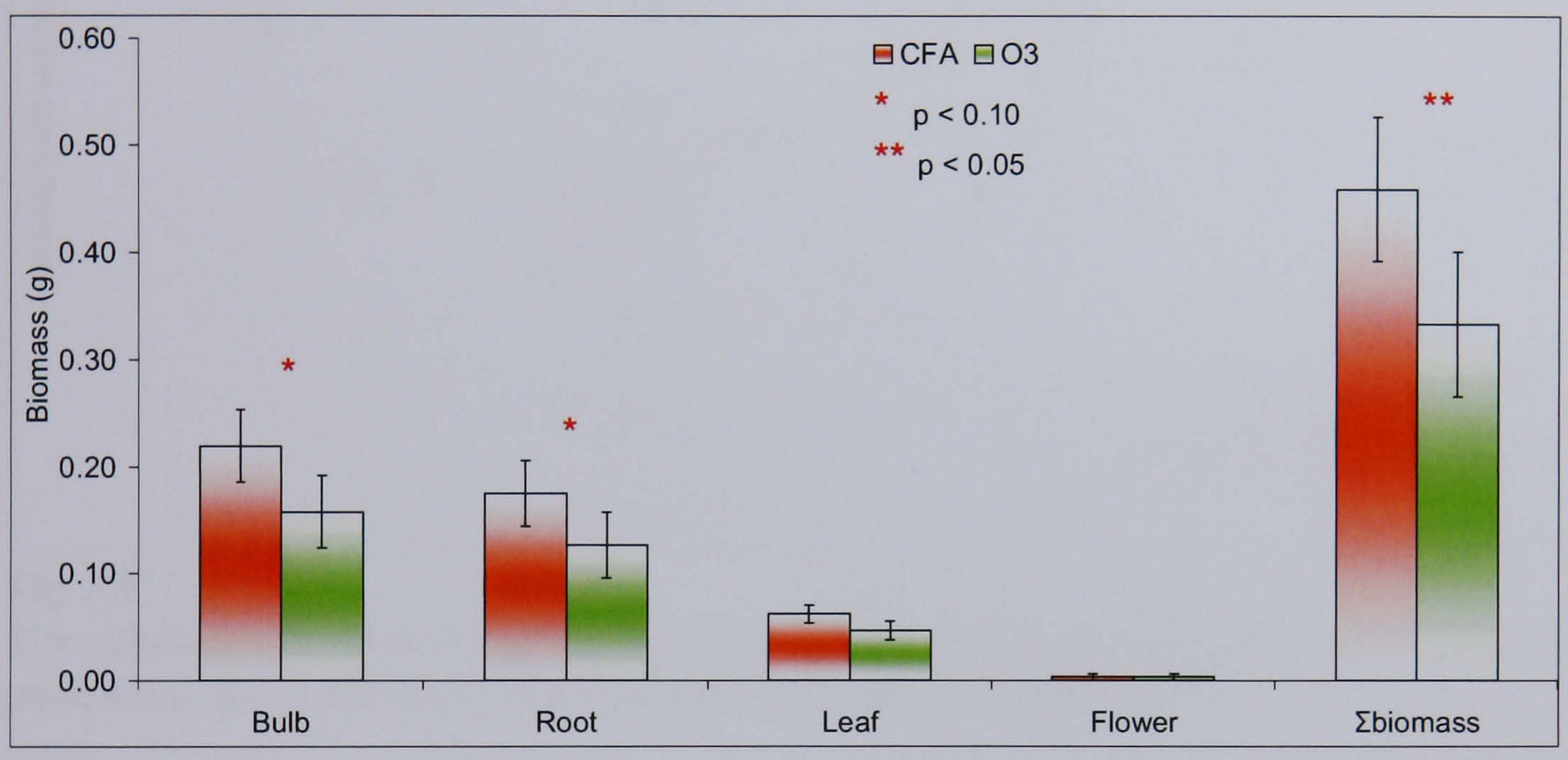


Fig 3.4
Live Biomass of *Allium ursinum* at the end of the experiment.
Error bars represent +/- 1s.e; where: * = $p < 0.10$ and ** = $p < 0.05$; Flower weight = 10 x weight (g)

Fig 3.5 shows the relationship between initial fresh bulb biomass and final dry bulb biomass. There is a good fit to the data in both treatments ($r = 0.678$; $p < 0.01$ in the control and $r = 0.51$; $p < 0.05$ in the ozone treatments). The slope of the fitted line is significantly lower in the ozone treatments, although the intercept is similar in the two treatments. The mean bulb weight in the CFA treatment was 0.22 ± 0.034 g and in the O3 treatment 0.16 ± 0.016 g ($t = 1.71$; $p < 0.10$; d.f. 33). Due to the significantly greater slope in the control treatments, the final dry weight of bulbs, produced by those bulbs with a higher initial wet weight

(between 0.5 and 1.5 g) were consistently higher than those in the ozone treatment. This suggests that ozone was a significant constraint on the growth and development of the larger, but not the smaller, bulbs.

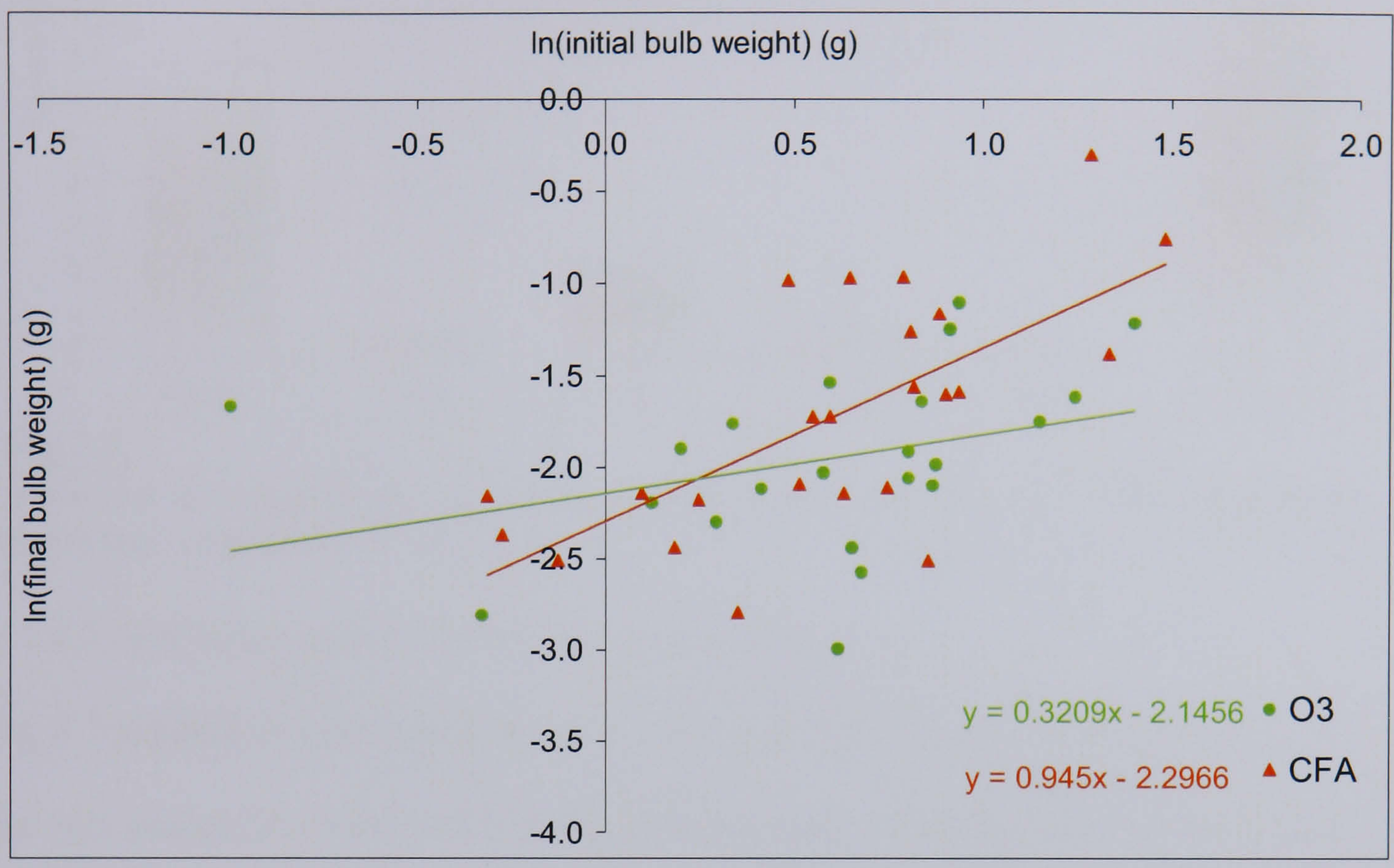


Fig 3.5
The relationship between initial wet bulb weight and final bulb dry weight in the two treatments for *A.ursinium* The plotted values are ln weight (g).

3.3.4 *Narcissus pseudonarcissus*

Fig 3.6 shows the final dry biomass values for various component parts of *N.psuedonarcissus*. Ozone consistently increases biomass in *N.psuedonarcissus* but no changes in biomass were significant (Table 3.3).

Fig 3.6
Final live biomass (g) for *Narcissus psuedonarcissus* at the end of the experiment. Error bars represent +/- 1 s.e.

3.3.4.1 Leaf Injury and Deformity in N.psuedonarcissus

Fig 3.7 summarises the extent of visible injury on the *N.psuedonarcissus* plants in the two treatments, with rank 3 being the most badly damaged (see Table 3.1). It is assumed that this injury and leaf distortion was caused by a fungal infection, likely to be *Fusarium spp*., to the plants. The symptoms the plants were exhibiting and the presence at the early stages of the experiment of a white and yellow coating to the soil and bulb surfaces suggest this type of infection.

The frequency of injured plants (21%) was significantly greater in the ozone treatment than in the control treatment (33%) and many more plants remained healthy in the control treatment. However, there was no evidence of the degree of injury in affected plants being significantly greater in the ozone treatment.

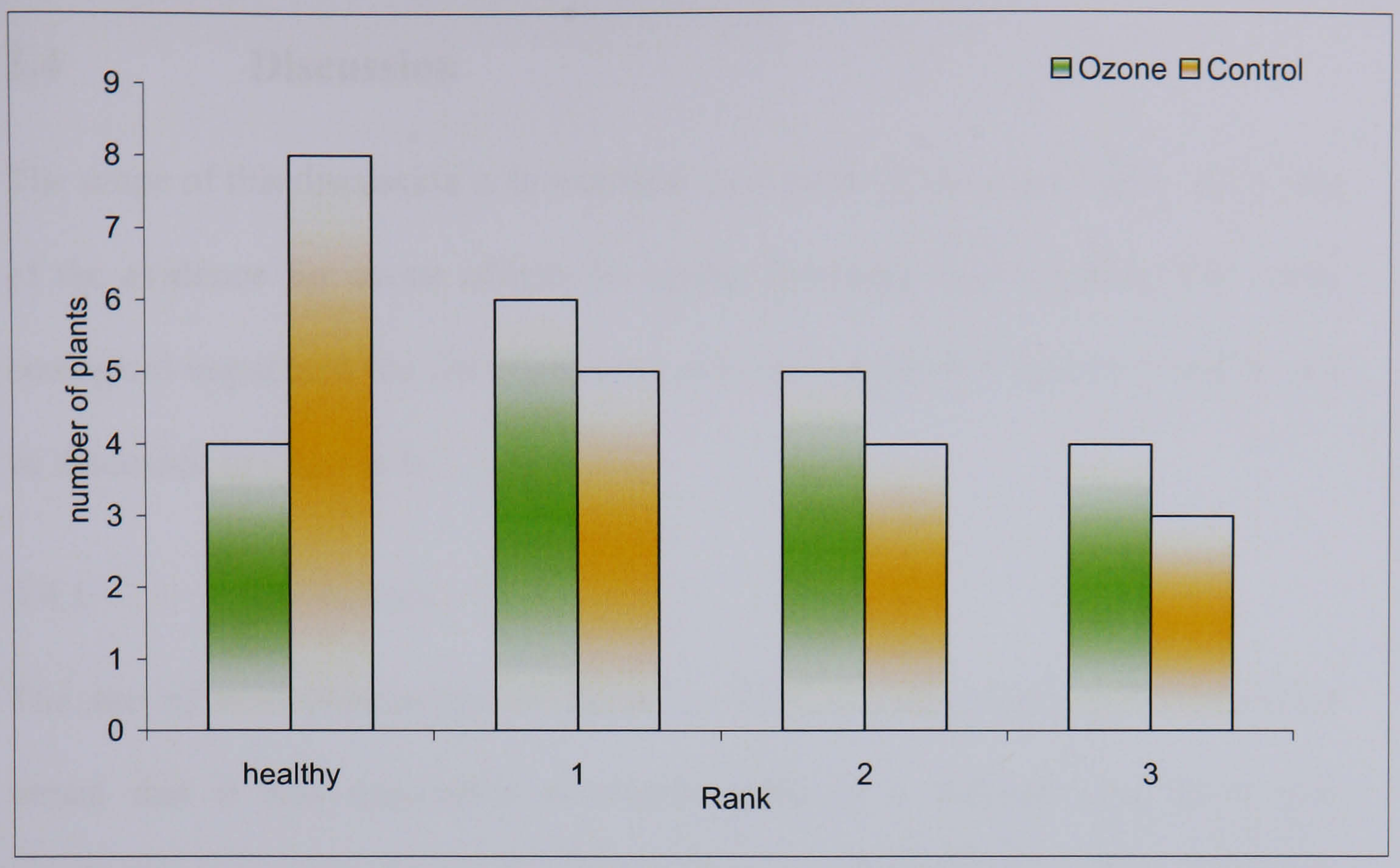


Fig 3.7
Number of plants showing different degrees of foliar injury in the two treatments for *N.psuedonarcissus.* Rank is based on the descriptions in Table 3.4.

3.4 Discussion

The scope of this discussion is to examine, in context of the experiment, the extent of the evidence for ozone effects on spring flowering bulb species. The wider ecological impacts of the findings, along with the results of Chapters 4 and 5, will be discussed in Chapter 6.

3.4.1 Limitations

The use of wild populations of bulbs for both *H. non-scripta* and *A.ursinium* meant that it was impossible to obtain bulbs of a uniform size. It is also impossible to account for previous life history of each individual. This inevitably led to considerable variation in final size of the experiment plants. Although initial bulb weight was used as a covariate to try to account for some of this variation, it was the fresh weight, and would have been affected by the water content of the bulbs.

Growth rate and final biomass of *H. non-scripta*, in particular, was low for both treatments and it could be said that transplantation and chamber conditions were detrimental to the growth of this species in this experiment. Open-top chambers may be more suitable for growth for these species, giving them more natural conditions for growth, although it may be the transplantation itself that is more stressful. It may be more appropriate to transplant intact turf samples without removal of bulbs, and to transfer these directly to chambers without disturbance. This method, however, would give an unknown quantity and quality of bulbs.

Given these limitations of the experiment, this study should only be considered as a preliminary assessment of the potential for ozone effects on these important species. These preliminary results, however, have encouraged a larger-scale open-top chamber experiment to be set up at the University of Newcastle. This experiment involves four levels of ozone exposure includes six species, the three used in the experiment described in this chapter, plus *Frittilaria meleagris* (Fritillary), *Ornithogalum umbellatum* (Star of Bethlehem) and *Tulipa sylvestris* (Wild Tulip). The results for the first year of this experiment (Peacock & Barnes, pers.comm.) show significant effects of moderate ozone exposure on visible injury and initial growth of the species; *H .non-scripta, O. umbellatum and T. sylvestris*. The other species are deemed too small for assessment at present. These have shown significant decreases at much lower exposure levels than used within this study; the Newcastle study has shown effects compared with: NFA (present-day spring ozone climate), NFA+20 nmol mol^{-1} O_3 (nominally akin to a 2075 UK spring ozone climate) and NFA+30 nmol mol^{-1} O_3 (nominally akin to a 2125+ UK spring ozone climate) treatments in which levels have not exceeded 80ppb.

There is no significant evidence from this experiment to suggest that ozone has a detrimental effect on the growth of *H. non-scripta* once plants had emerged. However, there was a suggestion that ozone could be affecting initial growth or sprouting of the bulb since the mean weight of unsuccessful bulbs was greater in ozone. Most of the differences relate to this variation in initial bulbs weight.

The failure of many of the *H. non-scripta bulbs* to grow may relate to the trauma from being transplanted. *H. non-scripta* populations become adapted to changes in

the canopy under which they grow and recovery following transplantation can take many years (Van Der Veken *et al.*, 2007). It is known that *H.non-scripta* have arbuscular mycorrhiza fungi associations with its roots (*e.g.* Helgason *et al.*, 1999; Helgason *et al.*, 2002) these play essential roles in uptake of nutrients for the plant especially phosphorous. In addition to this, it sheds it roots at the end of every growing seasons and regrows new roots in spring (Blackman and Rutter, 1954). Helgason *et al.*, (2002) suggest that new fungal partnerships are rebuilt in the growing season, and that this can be greatly impacted by soil disturbance. Taking this into account the impact of transplantation could have led to the lack of suitable growing conditions for the smaller bulbs which failed to grow in both treatments; larger bulbs can buffer these effects using their more ample supply of resources.

However, in the ozone treatment many of the larger bulbs failed to grow; it could be hypothesised that the ozone exposure is having effects on growth through the soil; since ozone does not penetrate the soil surface; it is difficult to identify the cause to the failure to grow of these larger bulbs.

In contrast, for *A.ursinium* there was significant evidence for negative effects of ozone on growth. Growth of this species was better in the chambers than *H. non-scripta*, however it was still slow and only three plants flowered. Reductions in bulb biomass suggest that there will be carry-over effects from ozone exposure on the following year's growth, which could lead to a large cumulative long-term effect of ozone.

Wilbourn *et al.* (1995), state that persistent effects of ozone might lead to poorer winter survival and decreased rates of nitrogen fixation. Wilbourn *et al.* (1995) reported a persistent effect of elevated ozone on stolon density in *Trifolium repens,* during an open air field fumigation study with cutting at regular intervals to stimulate silage production and grazing. Fumigalli *et al.* (2003), over a four year study on ozone sensitive and resistant *T.repens* clones exposed to ambient air, report a 60% reduction in yield of ozone sensitive clover, the percentage difference in yield between the resistant and sensitive clones was shown to increase over consecutive seasons (Fumagalli *et al.* 2003) demonstrating a carry-over effect of exposure on the following seasons growth.

There is very little to suggest that there are negative effects of ozone if any at all for *N. pseudonarcissus.* However there is evidence to suggest that ozone lowered the sensitivity to fungal infections. The use of ozone in the food industry as an antimicrobial agent suggests that the presence of large quantities of ozone should slow or kill any microbial growth (see review by Guzel-Seydim *et al.*, 2004). Furthermore, studies have shown that ozone exposure can lead to declines in *Fusarium* population size within wheat crops (Kottapalli, 2005) however these tend to be at considerably higher concentrations than present in the troposphere, Raila (2006) reported exposure concentrations of 700ppb.

The occurrence of more foliar injury on *N. pseudonarcissus* may suggest that some of this injury may be a direct result of ozone exposure. It may be the case that ozone damage had opened the tissues of these plants to allow a sheltered environment for the fungi to colonise within the plant; however this has not led to

any reductions in biomass. It seems that the interactions of plant disease and the effects of ozone require further investigations.

3.4.5 Conclusions

There is evidence from this first study to suggest that there are serious implications for spring flowering bulbs exposure to high concentrations of ozone. The effects of exposure caused reductions in biomass especially of the below ground parts of the plants which are often overlooked in exposure studies, but are crucial for the ecological success of these species. The lack of previous studies of ozone and spring flowering bulbs gives importance to this study's findings as foundation for further research. However the general unsuitability of the growing conditions had a negative impact on the growth of the plants. A longer term study is required to expand on this study and investigate ozone exposure on such species.

4. Chapter 4: Field observation of ozone concentrations and stomatal conductance in upland vegetation of the Yorkshire Dales.

4.1 Introduction

The laboratory experiments described in Chapter 2 and 3 demonstrate the potential for ozone to affect individual woodland ground flora species. However, the extent to which ozone actually affects these species in the field depends on a number of factors, including how the position of the species within the plant canopy modifies both ozone concentrations to which it is exposed, and stomatal conductance, which may influence the flux of ozone to sites of damage in the leaf. For example, lower ozone concentrations and reduced stomatal conductance might be experienced by woodland ground flora, because of ozone deposition to, and light interception by, the woodland canopy, and this may reduce the impact of ozone under field conditions.

There are few studies which have examined profiles of ozone within plant canopies. The two most recent and relevant studies suggest that ozone is reduced inside a canopy but still infiltrates plant canopies to a significant depth (Jaggi *et al*; 2006, Karlsson *et al*; 2006).

Karlsson *et al* (2006) observed the levels of ozone within and outside a Norway Spruce forest in Sweden. They found that compared to concentrations outside or above the canopies, ozone concentrations inside the forest were reduced by 3–8% during mid-day and 10–40% during night-time. Because of threshold effects, the

AOT40 index was reduced significantly, by 15–45% inside as compared to outside the forest.

Jaggi *et al.* (2006) examined exposure levels of grassland plants within a canopy at 7 points within a vertical profile (0.05, 0.10, 0.20, 0.30, 0.50, 0.90 and 1.50m); and clearly demonstrated how concentrations of ozone vary within a canopy's profile; the deeper within the canopy the lower the concentrations. The grassland community studied had two pronounced canopy layers: 0.25m and 0.50m, distinguished by the vertical distribution of grassland species. The mean ozone concentrations were reduced by 36% at the lower canopy level. In contrast to similar studies on ozone concentrations within other types of plant community, the results of Jaggi *et al.* (2006) show a steep gradient and less infiltration into the canopy, demonstrating the variability in natural communities.

Concentrations of ozone within the canopy layer were largely influenced by air movement in the studies of Jaggi *et al.* (2006) and Karlsson *et al.* (2006), and not by leaf area index or leaf angle distribution (Jaggi *et al.*; 2006). Norway spruce canopies are a lot denser than typical broadleaved woodland; broadleaved woodland, with a more open canopy structure could be expected to have more air movement and thus a greater degree of penetration of ozone into the field and shrub layer.

In order to further investigate these phenomena, field measurements were carried out during the summer of 2005 at two sites, the woodland site at Grass Wood and the grassland site at Colt Park, Ingleborough NNR. The canopy structure within

woodland creates many different types of microhabitats for the many species adapted to this habitat, and therefore measurements were made at several locations within the wood.

4.2 Methods

4.2.1 Grass Wood

4.2.1.1 Study Area

Measurements of ozone concentrations were made inside the wood, and were compared with concentrations outside the wood. Fig 4.1 shows a map of Grass Wood and its surrounding area, 'y' indicates the position of an ozone monitor outside the woodland perimeter and the blue zone indicates the areas inside of the woodland where the ozone monitor was positioned during the measurement campaigns. The black shaded areas are the zones in which sampling took place over an uphill gradient, starting on Day 1 at the nearest black area to 'y' and working further into the wood. This area of Grass Wood was selected for study due to it being situated on an easy access route and containing the species which were targeted for conductance measurements. Fig 4.2 shows photographs of each of the sampling sites on consecutive days.

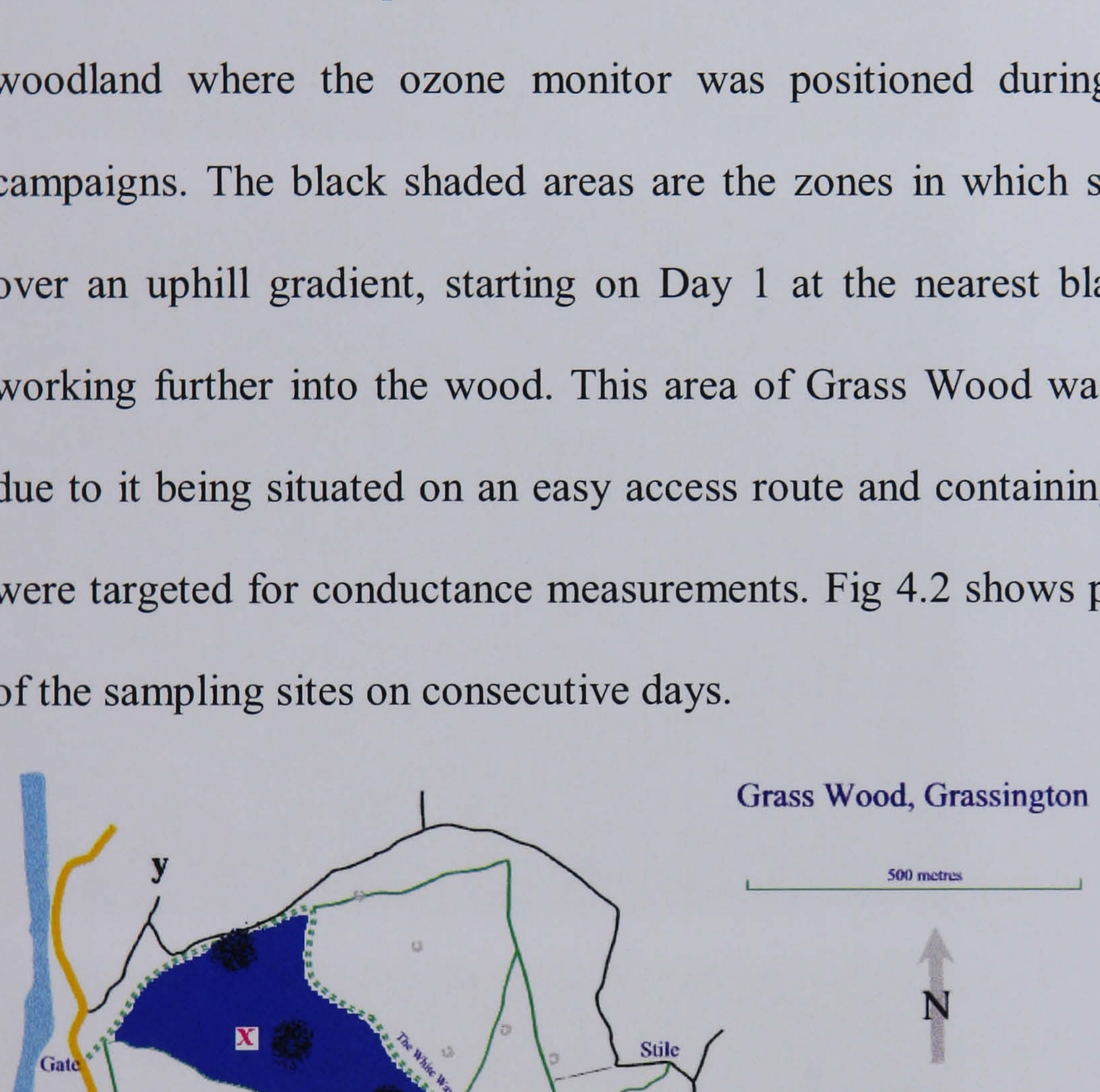


Fig 4.1
Map of Grass Wood Indicating Study Area in Blue.

Original in colour

(a)

(b)

(c)

Fig 4.2
Photographs of the study areas within Grass Wood (a) Day 1, (b) Day 2, (c) Day 3.

4.2.1.2 Sampling dates

Two periods of sampling took place at Grass Wood; 26 – 29 May 2005 and 8 – 12 June 2005. Sampling in May included ozone concentrations, climatic variables, and stomatal conductance. The period in June included ozone concentrations and climatic variables only due to time constraints and repair to the Cirras-I.

4.2.1.3 Target Species (27-29 May 2005)

Three zones within this area were selected for study on sequential days. The first area contained a large population of *Allium ursinium*, a spring ephemeral species which showed some sensitivity to ozone when grown alone (see Chapter 3). The second zone was mainly a mix of *Mercuralis perrenis* and *Viola riviana* with occasional/rare other species. The final zone, which was at the top of a plateau within Grass Wood, was mainly *Convallaria majalis* and *Viola riviana;* no *M.perrenis* was present. Table 4.1 gives the details of the target species for each day and zone. These species were mainly selected due to their leaf size being big enough for the infra-red gas analyser (IRGA) cuvette and due to their dominance within the woodland areas.

Table 4.1
Summary of Target Species in Grass Wood

	Day 1 / Zone 1 27/05/05	Day 2 / Zone 2 28/06/05	Day 3 /Zone 3 29/06/05
Allium ursinium	X		
Mercuralis perennis		X	
Viola riviana		X	X
Convallaria majalis			X

4.2.1.4 Ozone and Meteorological Recording

A portable ozone monitor (Model 202 Ozone Monitor; 2B Technologies, Colorado, USA) was set up at point 'y' (Fig 4.1) alongside a Photosynthetically Active Radiation (PAR) sensor (Skye instruments, Quantum (SKP 215)), and temperature and humidity sensors (Grant Instruments, miniloggers.). A PTFE sample line for ozone was set at a standard 2m height. The site was approx. 500m away from the first sample zone on the edge of the woodland; it was situated in an adjacent field, next to a stone wall. The ozone sampling line was raised above this and all other sensors were placed on top of the wall to avoid the shade effect of the wall.

During the recording period 26 May 2005 – 29 May 2005, a second 2B ozone monitor was set up and moved at 18:00 to be located at each sampling zone, ready for the next day, within area 'x' (Fig 4.1) of Grass Wood. The PTFE sample line for this monitor was placed at the height of the woodland ground layer vegetation, approx 5-10cm above ground level. Temperature and humidity sensors were also set alongside the monitor at ground level.

A second period of ozone monitoring took place between 8 June 2005 and 12 June 2005. During this time the woodland monitor was stationary for the entire period and placed centrally within zone 'x' (Fig 4.1); the same locations were used as before for zone 'y'. No meterological (temperature and humidity) measurements were made due to failure of the miniloggers.

The 2B ozone monitors were cross-calibrated against each other and an external standard (Dasibi, 1003-AH UV Photometer) prior to all installations at the field sites and when returned to the lab. The light sensors and miniloggers were also checked for consistency between them. Data was logged electronically by a Squirrel data logger (Grant Instruments).

4.2.1.5 Stomatal Conductance and Photosynthesis

A Cirras-I infra-red gas analyser (PP-systems) was used to measure stomatal conductance of the target species. The Cirras-I was calibrated and checked prior to all field work. Individual leaves from each species were recorded over a 10 minute period and these results were averaged. A different leaf was selected for each reading and the leaf completely filled the cuvette. Where two species were observed together, the species were alternated; e.g. species A for 10 minutes then species B for 10 minutes.

4.2.2 Ingleborough NNR

4.2.2.1 Study area

A grassland site close to Colt Park Wood within Ingleborough NNR was chosen for this study. Fig 4.3 shows a photograph of the hay meadow.

Fig 4.3
Photograph of upland hay meadow at Ingleborough.

4.2.2.2 Sampling Dates

Sampling at Ingleborough took place on two dates; 22-23 June 2005 and 8-9 July 2005.

4.2.2.3 Target Species

The hay meadow had large populations of *Caltha palustris*, which had large leaves suitable for use with the Cirras-I cuvette. This species was also selected because it was being studied by the Newcastle University team under the same research programme. The hay meadow was particularly diverse but all other species had small leaves unsuitable for use with the Cirras-I cuvette.

4.2.2.4 Ozone concentrations and meteorological variables

Ozone levels were recorded at the top of the *C.palustris* canopy and simultaneously at the base of the canopy. The canopy height reached 30cm and the lowest leaves within the canopy were at about 5cm. A large patch of *C.palustris*, approx. $5m^2$ in size, was used for this study and all readings were taken from within this zone. Two cross calibrated 2B portable ozone monitors were used to measure levels of ozone above and within the plant canopy. Weather data was recorded at this site at English Nature's own weather station within the same field however, this was not within the canopy.

4.2.2.5 Stomatal Conductance

As before, Cirras-I infra-red gas analyser was used to record stomatal conductance for *C.palustris*. This was done by selecting leaves from above and within the canopy alternately and recording over a 2 minute intervals over a 10 minute period from early morning to late afternoon. A different leaf was selected for each reading.

4.3 Results

4.3.1 Grass Wood

4.3.1.1 26 – 29 May 2005

Figs. 4.4 (a) and (b) show the hourly mean air temperature and relative humidity for the period from 21:00 on 26/05/05 to 10.00 on 29/05/05 at the different measurement points inside the woodland and at the stationary point outside the woodland. Temperature and relative humidity are similar inside and outside the wood. The biggest differences were the lower values of temperature and humidity outside the woodland at night.

Fig 4.4 (c) shows the hourly mean concentrations of ozone inside and outside the woodland; unfortunately there are gaps in the data due to of technical faults. Fig 4.4 (d) shows the section of data for which the two monitors were both recording without fault. During this recording period, the data shows that within the woodland the ozone levels are overall similar to those outside, but that the diurnal variation in ozone concentration is lower inside the woodland. Hence, higher peak concentrations of ozone occur during the day outside the woodland. However at night, levels of ozone are higher within the woodland than those recorded outside, with the rate of decrease in ozone concentrations in the second half of the day clearly being faster outside the woodland than inside.

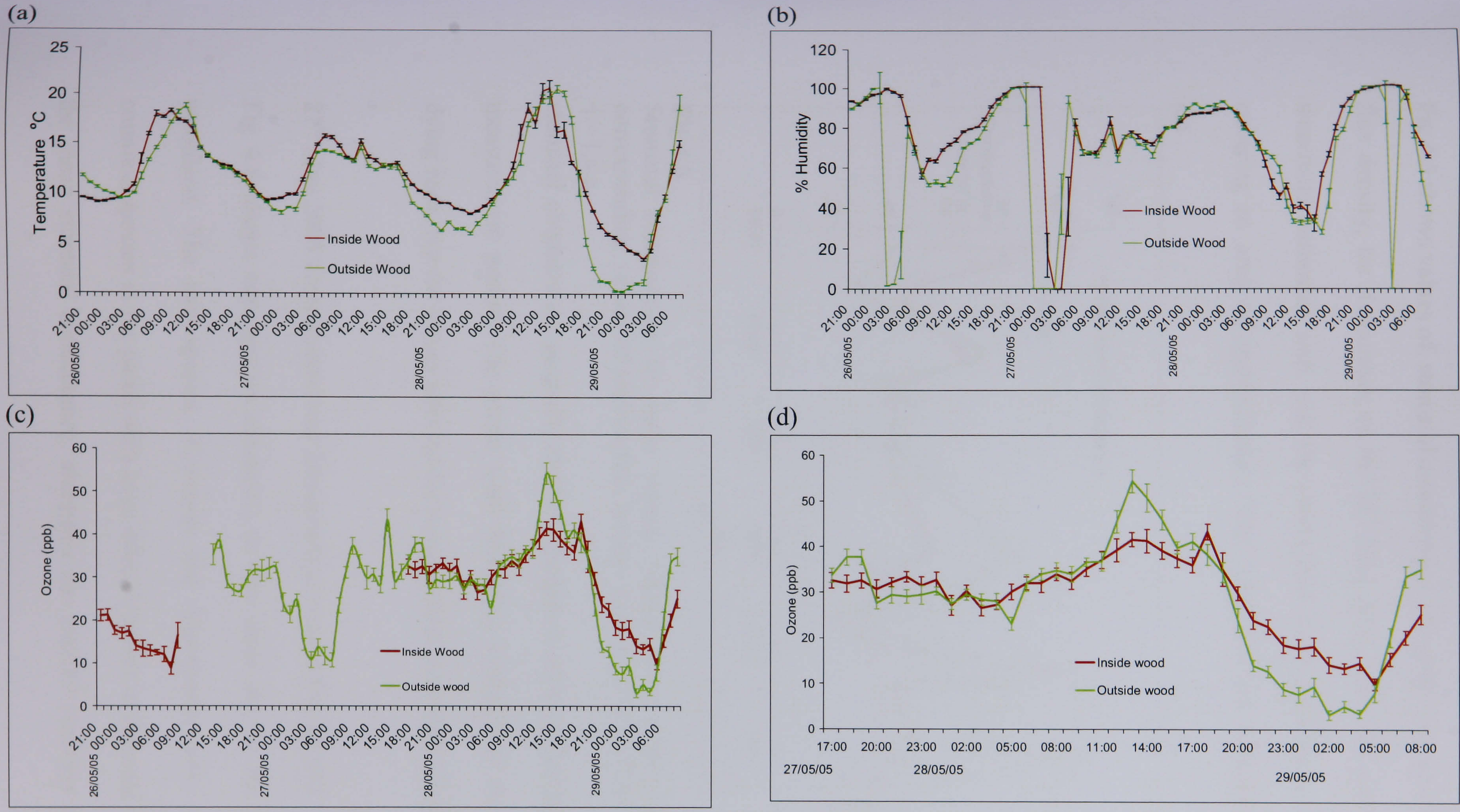


Fig 4.4
The hourly mean (a) air temperature, (b) relative humidity (c) ozone concentration for the entire period and (d) ozone recorded between 27th and 29th May 2005, during the first field campaign in Grass Wood

26 May 2005 - *Allium ursinium*

Fig 4.5 shows values of: stomatal conductance, alongside measurements of photon flux density, for *A.ursinium* on the 26th May 2005. This site was parallel to the woodland perimeter wall, and the plants here were growing in a monoculture covering an area of approximately $30m^2$ (Fig 4.3 (a)). Changes in values of

(a)

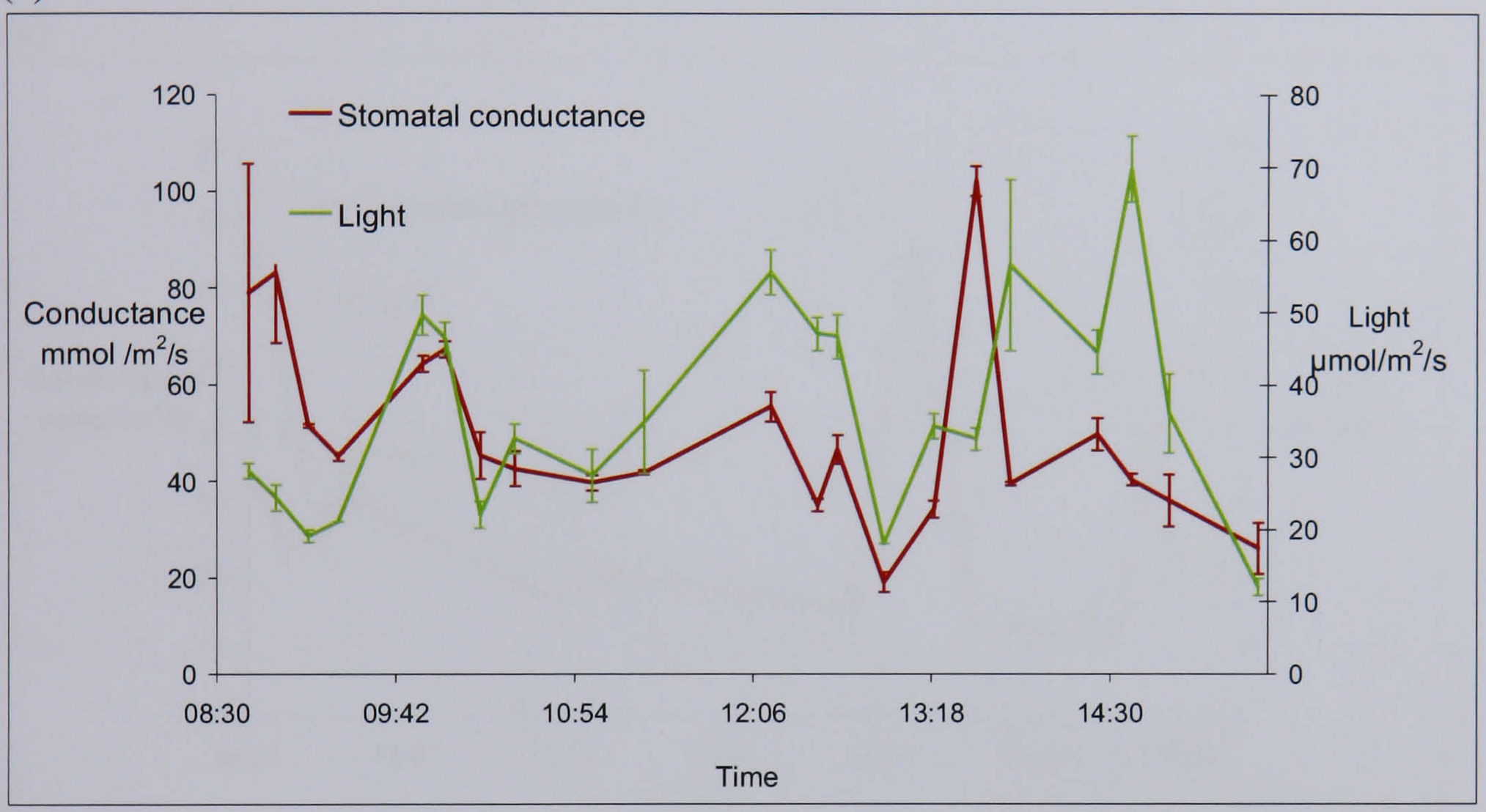


Fig 4.5
Stomatal conductance to water vapour (mmol m^{-2} s^{-1}) for *A.ursinium* with corresponding values of photon flux density (µmol m^{-2} s^{-1}). Error bars represent +/- 1.s.e.

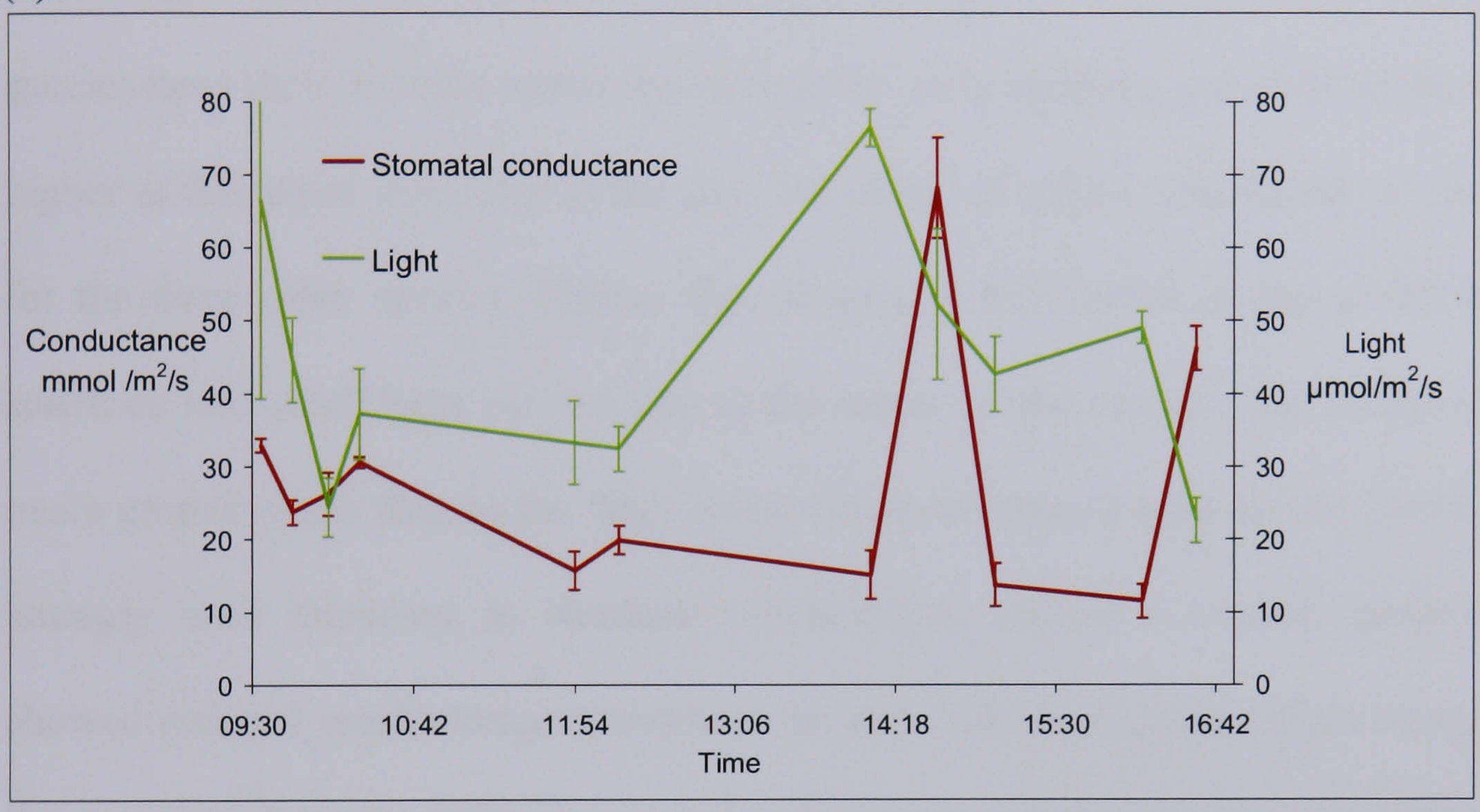
stomatal conductance generally tracked changes in photon flux density over the measurement period. The plants were becoming senesced as the flowers were dying back, but the leaves selected for measurement were fully green.

27th May 2005 *Viola riviana* and *Mercuralis perennis (Fig 4.3 (b))*

Fig 4.6 shows two species measured on the same day in the same zone of woodland. The two species, *V.riviana* and *M.perennis,* were the two most common species in the patch with large enough leaves to measure. Fig 4.6 shows the mean stomatal conductance, alongside the parallel readings of photon flux

density for *M.perrennis*, and *V.riviana.* Air temperatures in this area of the woodland were low; air temperatures varied between 10°C and 15°C during the measurement period and values of photon flux density did not exceed 80µmol m^{-2} s^{-1}, levels which are comparable to those for *Allium* (Fig 4.5). As for Allium, the measured values of stomatal conductance in both species were low, ranging from 50-100 mmol m^{-2} s^{-1}.

(a)



(b)

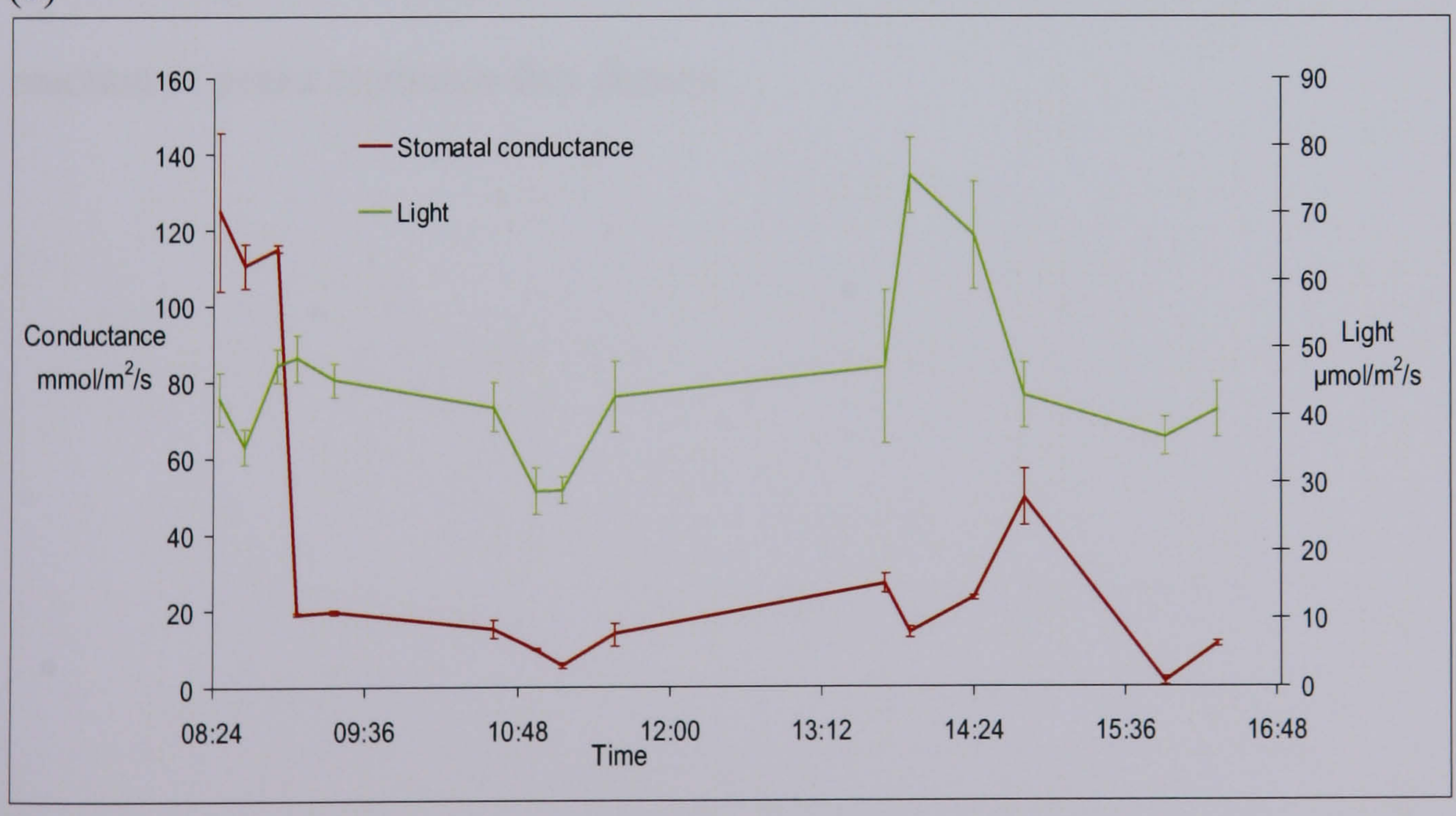


Fig 4.6

Stomatal conductance to water vapour (mmol m^{-2} s^{-1}) (a) *V.riviana* and (b) *M.perrenis* with corresponding values of photon flux density (µmol m^{-2} s^{-1}). Error bars represent +/- 1.s.e.

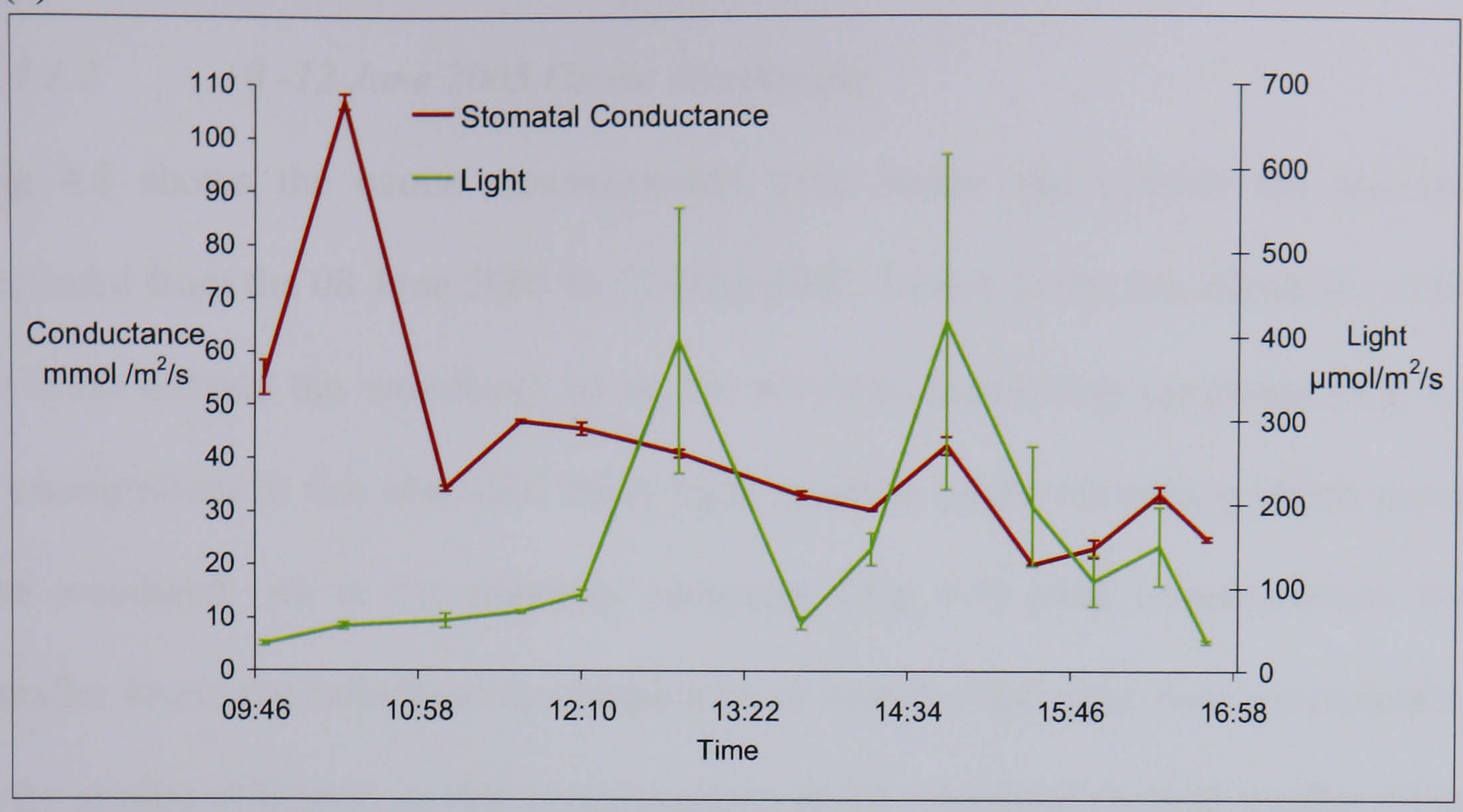
As for *Allium*, stomatal conductance was reactive to changes in photon flux density in both species, with delayed increases in conductance following peaks in photon flux density.

28 May 2005 *V.riviana* and *Convallaria majalis*

Fig 4.7 gives the data taken from the higher region of the wood on the 29th May 2005; Fig 4.7 (a) shows data for *V.riviana* and Figs 4.7 (b) for *C.majalis*. Both species have their stomata relatively open in the early morning and conductance is higher at this point than later in the day; the range of values was similar to those for the three other species. Photon flux density in this region of the wood was scattered into small light patches due to the nature of the canopy, and so showed much greater peaks than in the other areas. However, these peaks did not correlate strongly with increases in stomatal conductance; indeed *V.riviania* generally showed reduced conductance throughout the day with the highest values being in the morning. For *C.majalis*, in contrast, stomatal conductance showed a clearer reaction to peaks in photon flux density.

Original in colour

(a)



(b)

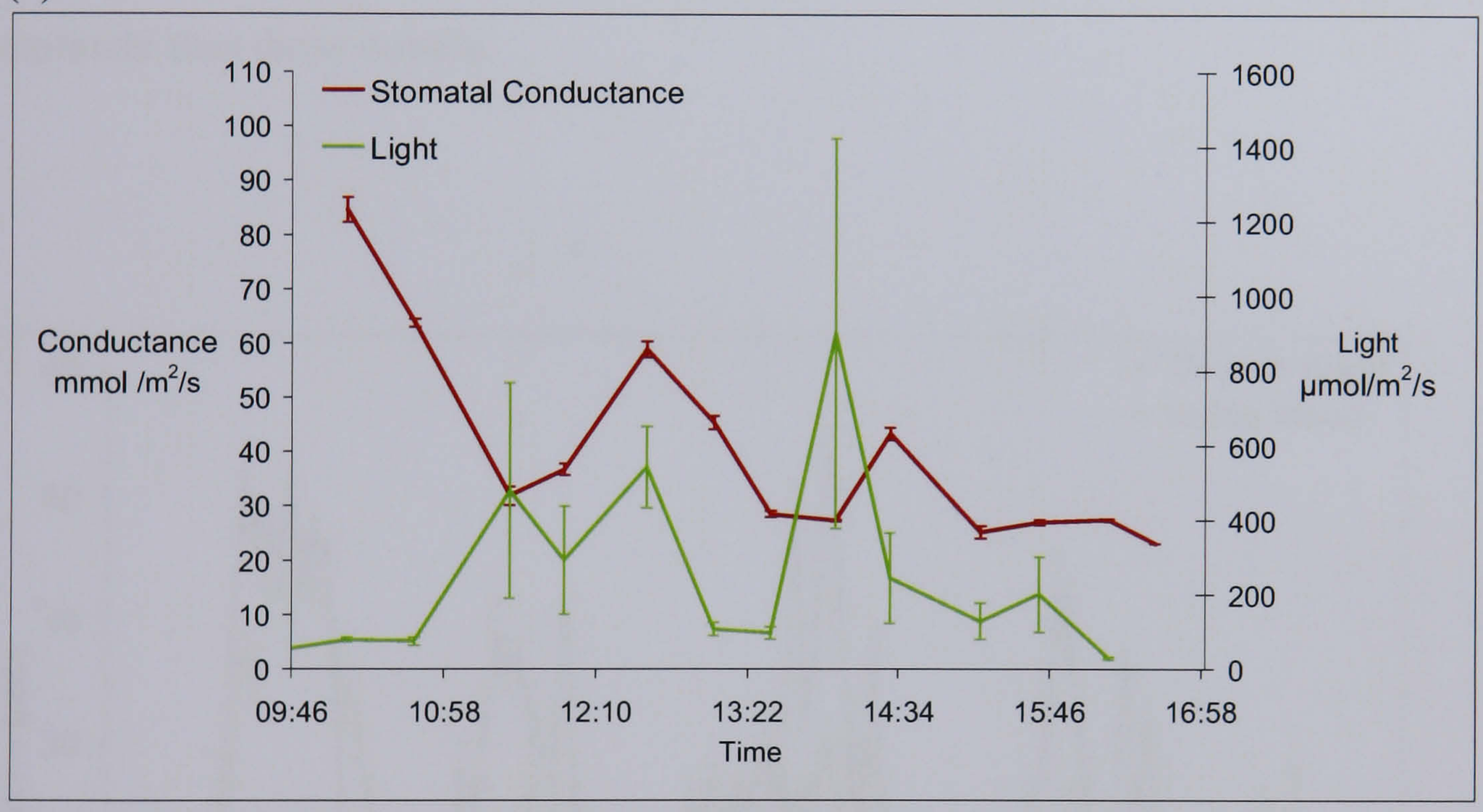


Fig 4.7

Stomatal conductance to water vapour (mmol m^{-2} s^{-1}) ((a) *V.riviana* and (b) *C.majalis* with corresponding values of photon flux density (µmol m^{-2} s^{-1}). Error bars represent +/- 1.s.e.

4.3.1.2 8 -12 June 2005 Ozone Monitoring

Fig 4.8 shows the ozone measurements from inside and outside the woodland recorded from the 08 June 2005 to 12 June 2005. Levels in the woodland are similar to those outside the woodland, as in the previous monitoring campaign (Fig 4.4). Concentrations at this site were fairly high, reaching peaks exceeding 60ppb outside the woodland. As in the previous campaign (Fig 4.4) peak concentrations were smaller inside the woodland in comparison to outside, but night-time concentrations were similar or higher, so that concentrations in the woodland showed smaller diurnal amplitude than those outside.

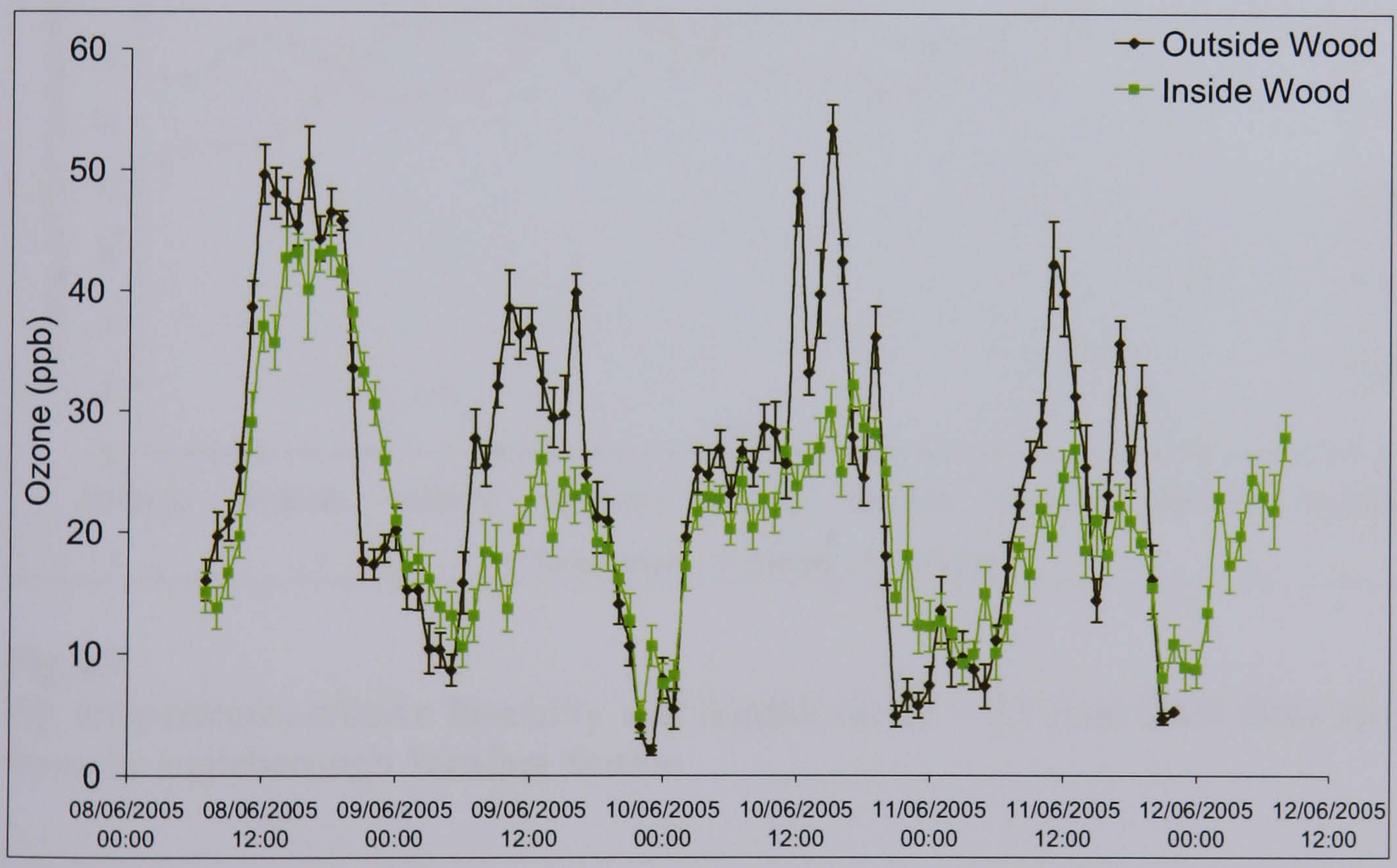


Fig 4.8
Mean hourly ozone concentrations (ppb) recorded from 08/06/05 – 12/06/05 inside and outside of Grass Wood Error bars represent +/- 1.s.e.

4.3.2 Ingleborough NNR

4.3.2.1 22-23 June 2005 Ingleborough

Meteorological Data

The data recorded from English Nature's weather station is shown in Fig 4.9; this includes air temperature, relative humidity and rainfall; from the start of 22nd June to the end of 23rd June 2005. As mentioned in the Methods section (Section 4.2.) the altitude of the weather station was slightly higher than the field study site. The maximum temperature recorded by the weather station was 15°C in the shade. The weather on these two days was bright but cloudy with a strong breeze.

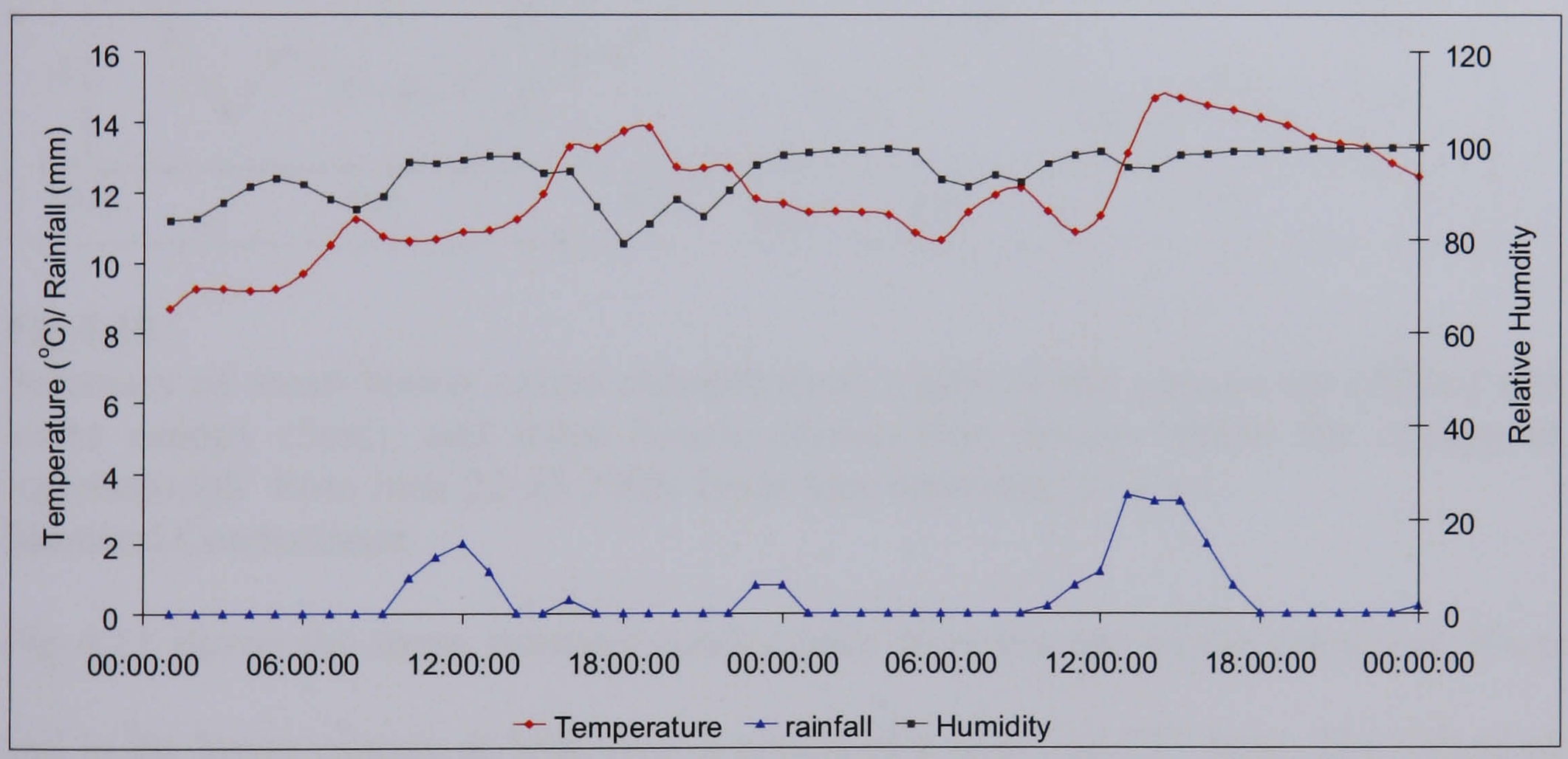


Fig 4.9
Air temperature, relative humidity and rainfall on 22 – 23 June 2005 Data are taken from the Ingleborough Weather Station

Fig 4.10 shows the mean hourly photon flux density and mean hourly ozone concentration in the field site at Ingleborough on the 22-23 June 2005. Ozone concentrations were measured, in areas of C. *palustris,* at the top of the canopy, at a height of 30cm, and from within the canopy, at a height of 5cm. The period captured

here is from 22.00 on the 22/06/05 to 18.00 on 23/06/05. Fig 4.10 shows that, during the day, mean levels of ozone were generally between 20-50ppb at the canopy top. Concentrations of ozone in the lower canopy were comparable to those above the canopy between 06.00 and 09.00 on 23rd June, but after 09.00, they fell rapidly, and were consistently much lower than those above the canopy.

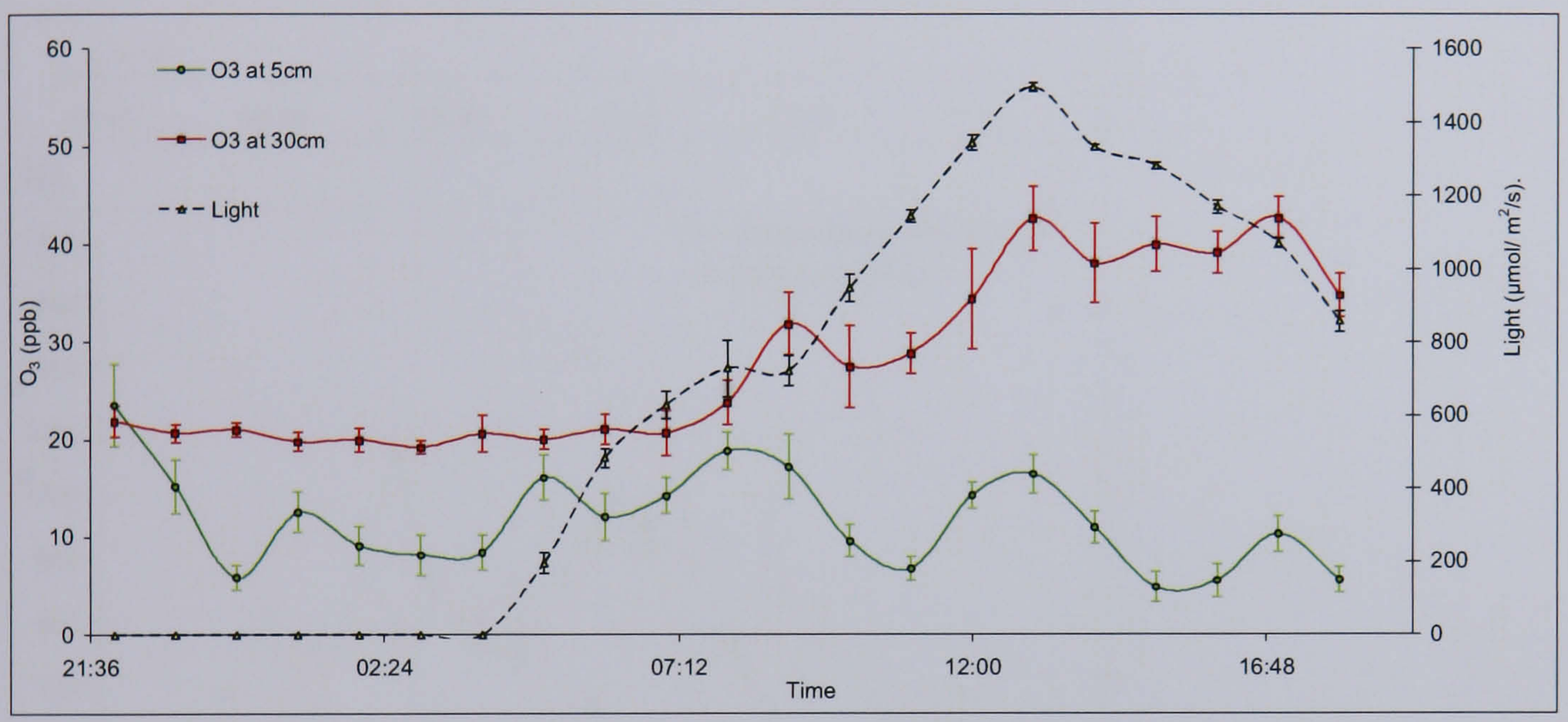


Fig 4.10
Summary of mean hourly ozone concentrations (ppb) in the canopy top (30cm) and lower canopy (5cm), and mean hourly photon flux density above the canopy at Ingleborough from June 22-23 2005; Error bars represent +/- 1 s.e.

Stomatal Conductance

Fig 4.11 shows the mean stomatal conductance from the top of the canopy at 30cm and in the lower canopy at 5cm, over a period of 9 hours on 23rd June. The values of stomatal conductance in *C.palustris* were relatively high, and ranged from a mean of 500 – 1400 mmol m^{-2} s^{-1}, Fig 4.10 (c) shows the conductance at 30cm and 5cm together; overall the values at the two heights are quite similar. This is despite the lower values of photon flux density at 5cm. On occasions, the photon flux density at this height was very low, but this seemed to have little effect on the stomatal conductance.

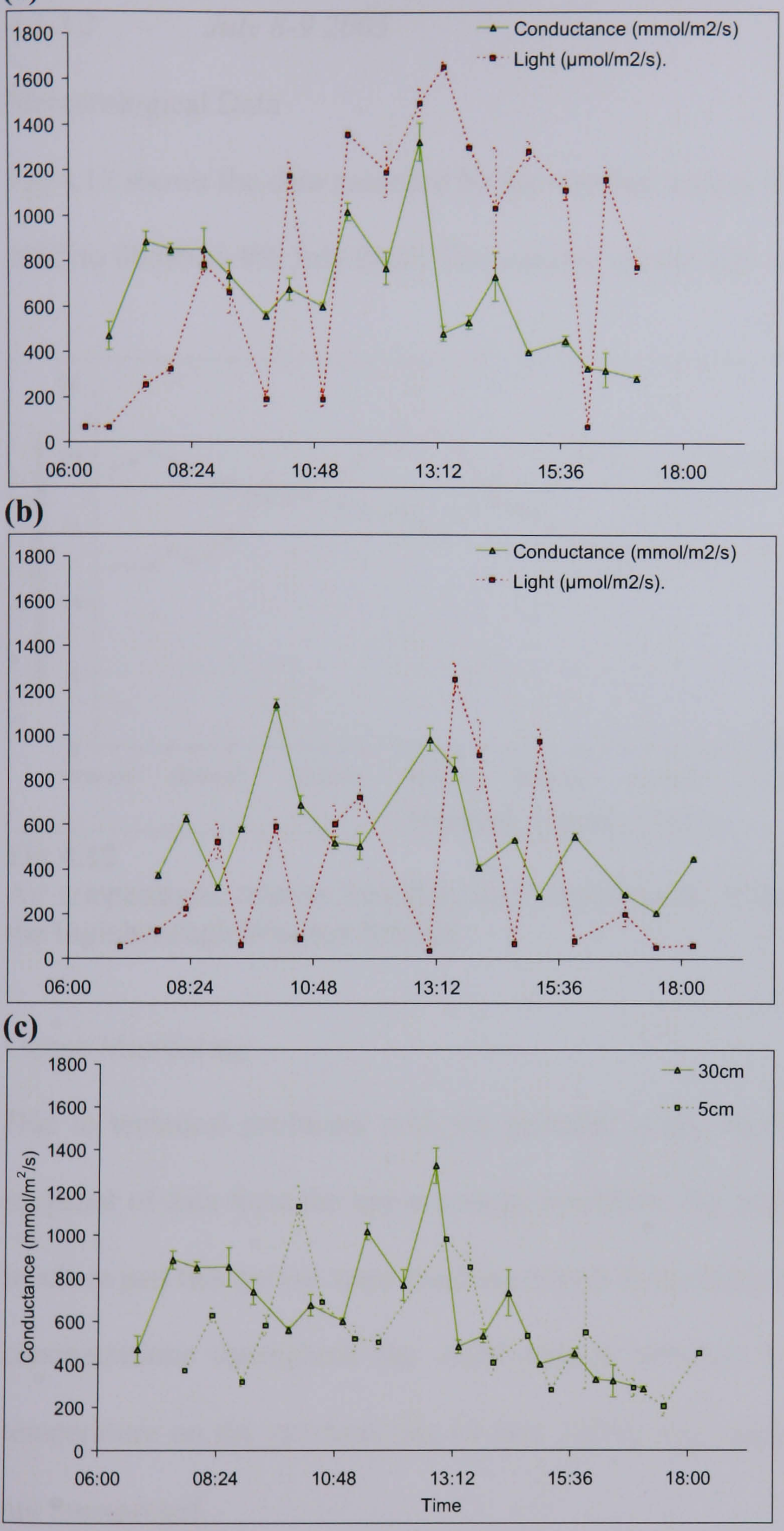


Fig 4.11

Stomatal conductance to water vapour (mmol m^{-2} s^{-1}) over a 9 hour measurement period on 23rd June, 2005, for *C.palustris* (a) at the canopy top (30cm) and (b) lower in the canopy (5cm) with corresponding values of photon flux density (µmol m^{-2} s^{-1}); and (c) stomatal conductance to water vapours for 30cm and 5cm together. Error bars represent +/- 1.s.e.

Original in colour

4.3.3.2 July 8-9 2005

Meteorological Data

Fig 4.12 shows the data recorded by the weather station from 00:00 hours on 8th July 2005 to 00:00 on 9th July 2005. The weather on this day was hot, sunny and humid.

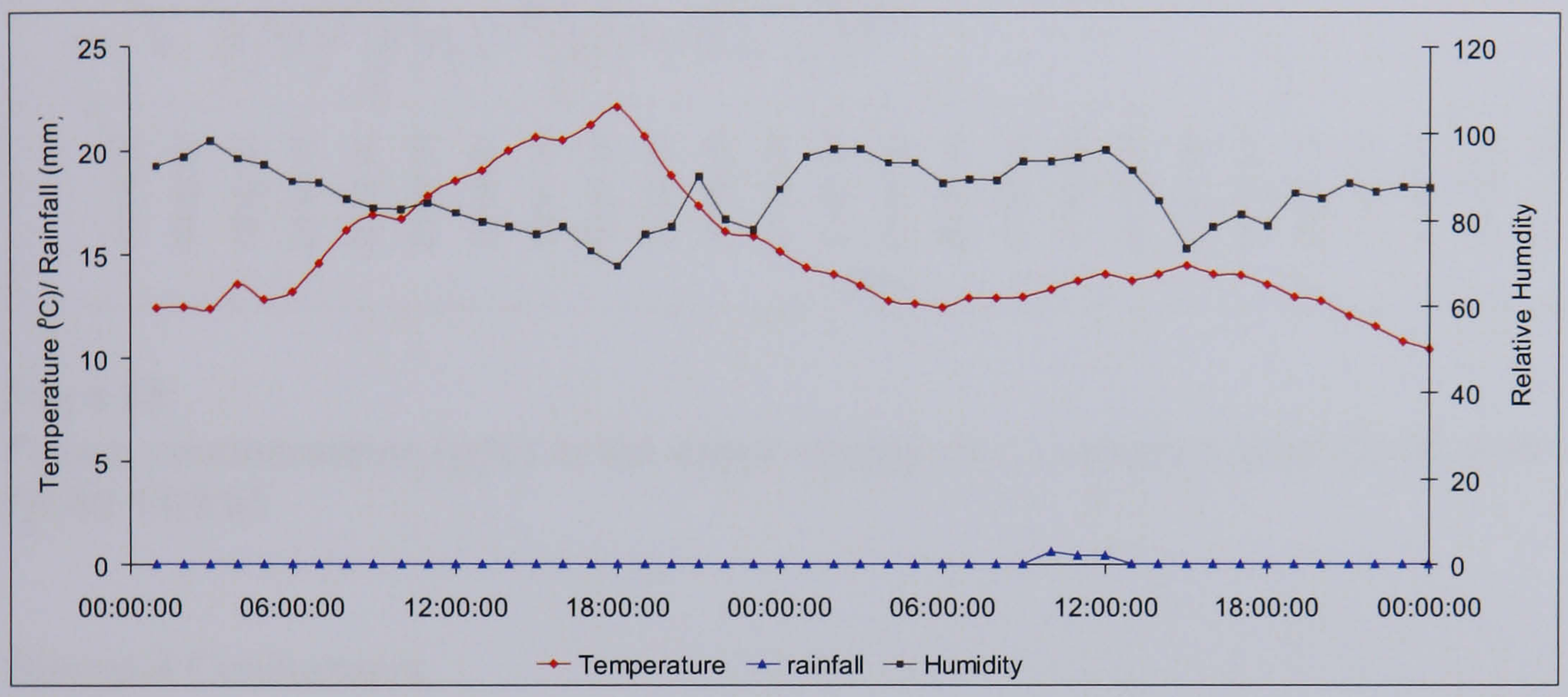


Fig 4.12

Air temperature, relative humidity and rainfall on 8 - 9 July2005 Data are taken from the Ingleborough Weather Station

Ozone Monitoring

Due to technical problems with the portable ozone monitors, there is only a small snapshot of data from the upper canopy available; this is shown in Fig 4.13. However levels in just this period were reaching 60ppb in the early hours of the morning, while concentrations throughout the night ranged between 0 and 30ppb. With a high temperature on the previous day (8 July 2005), high early morning ozone levels are not unexpected.

Original in colour

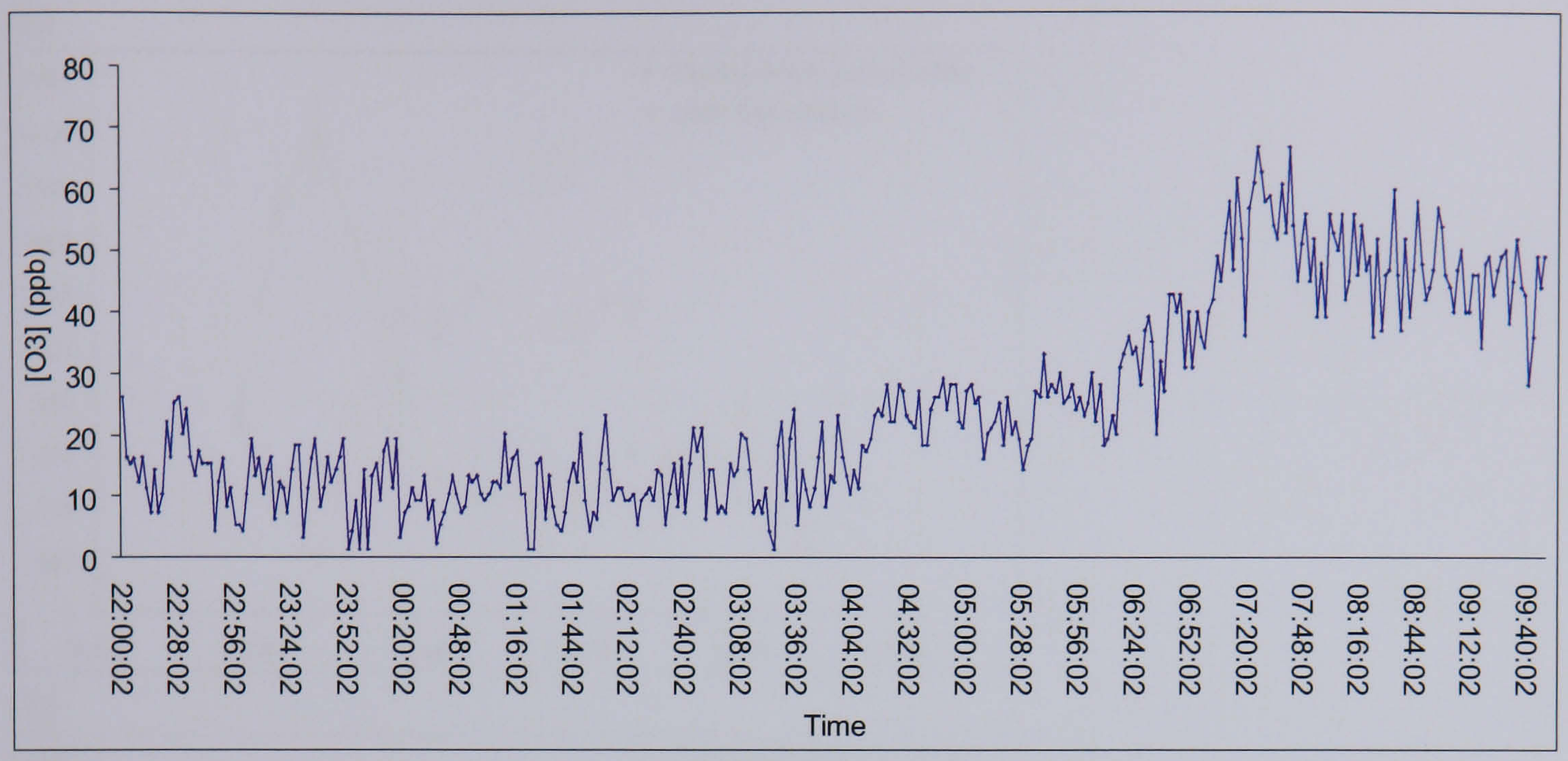
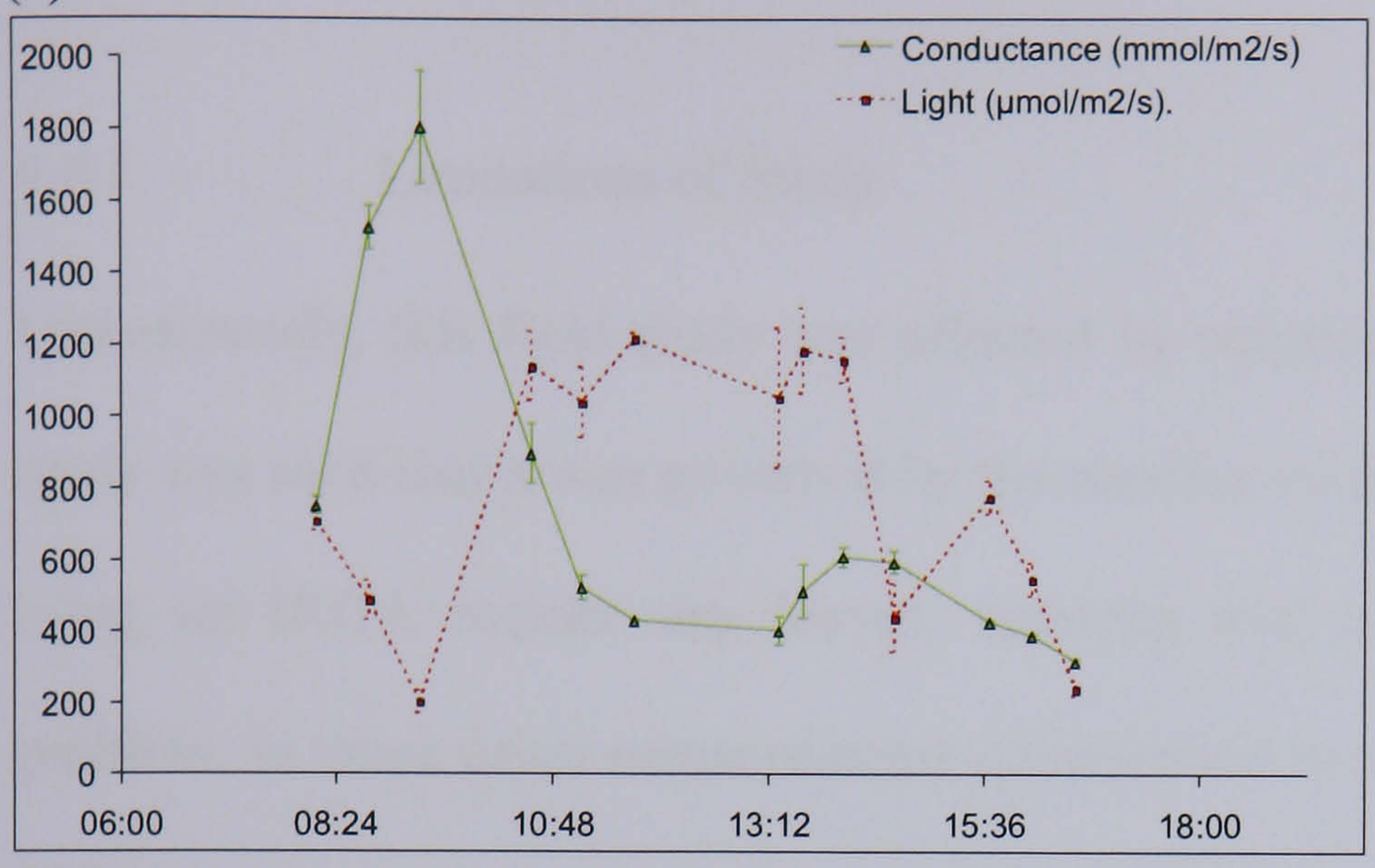


Fig 4.13
Ozone concentrations (ppb) in the upper canopy of *C. palustris* from 22:00 8/09/05 – 09:40 9/09/05

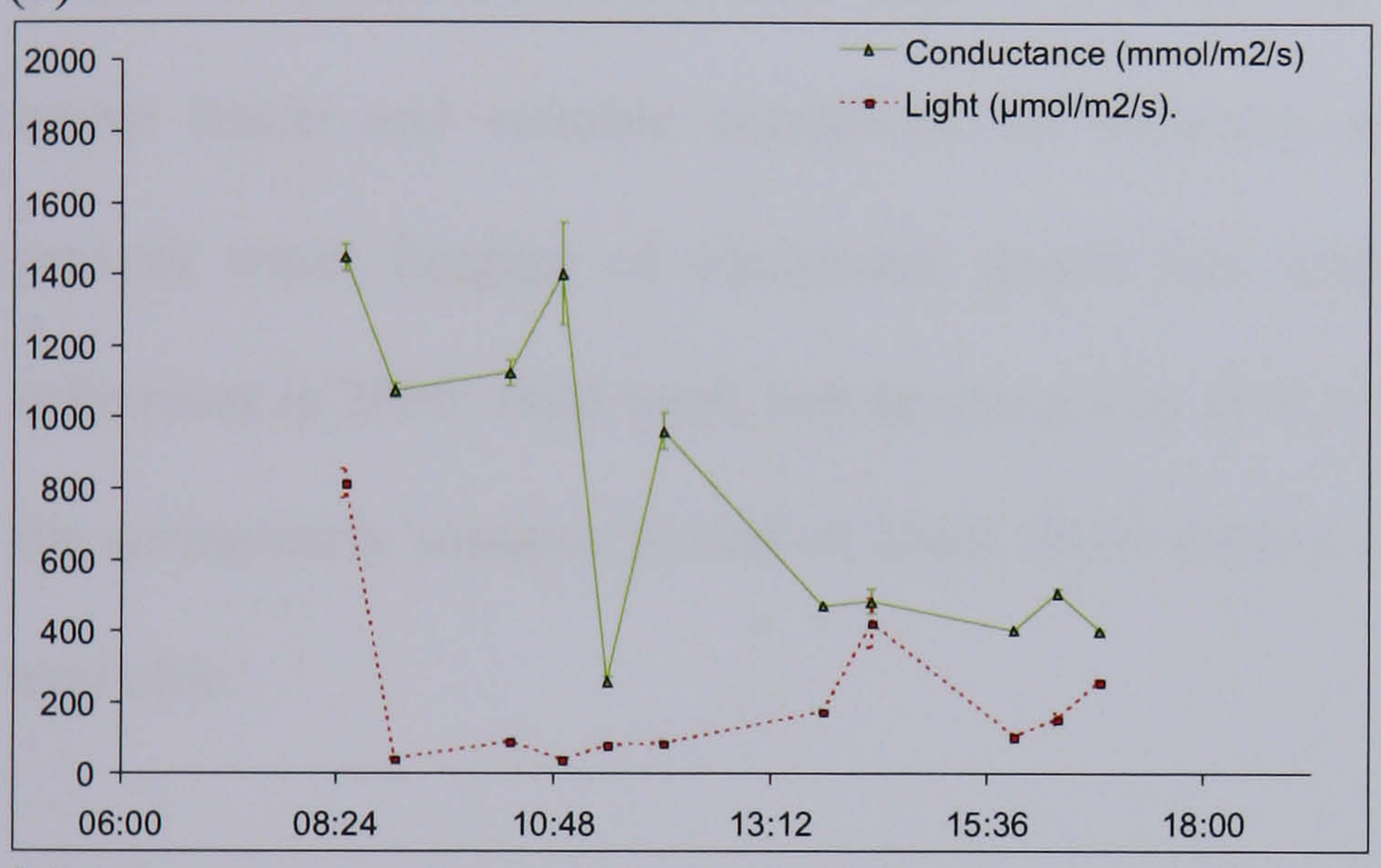
Stomatal Conductance

Fig 4.14 shows the mean stomatal conductance in (a) the upper canopy, 30cm and (b) the lower canopy, 5cm; alongside paired measurements of photon flux density. In the upper canopy (Fig 4.14(a)) stomatal conductance was high in the early morning and quickly reduced throughout the day to be between 500-600 mmol m^{-2} s^{-1}. Conductance in the lower canopy was much the same as the upper, with high conductance in the early morning and stabilising around 500 mmol m^{-2} s^{-1} from 11:00 onwards (Figure 4.14(b)). Values of stomatal conductance in both the upper and lower canopy seem unrelated to any changes in photon flux density, and the much lower values of photon flux density recorded at 5cm. did not seem to cause lower values of stomatal conductance.

(a)



(b)



(c)

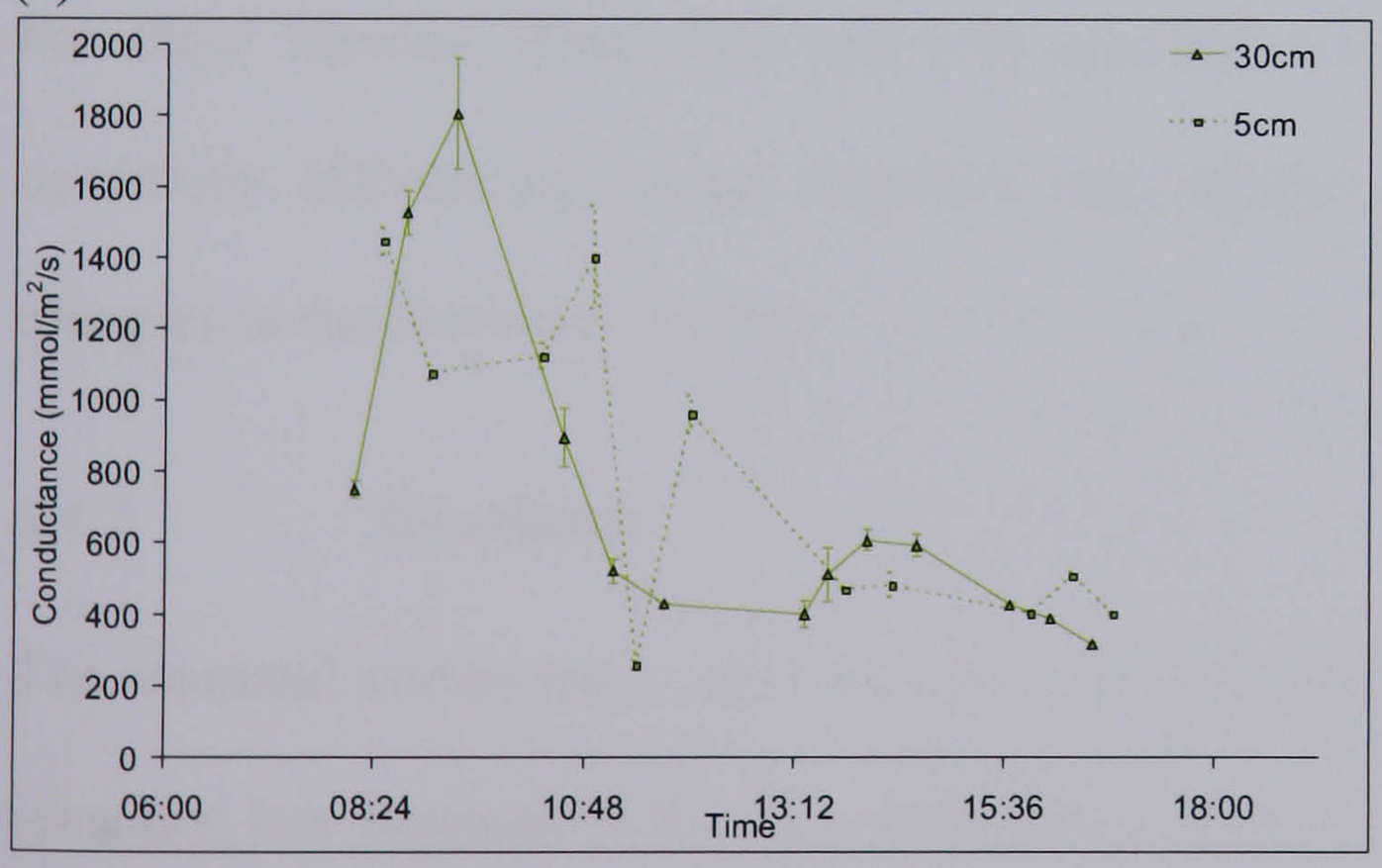


Fig 4.14

Stomatal conductance to water vapour (mmol m^{-2} s^{-1}) over a 9 hour measurement period on 9th July, 2005, for *C.palustris* (a) at the canopy top (30cm) and (b) lower in the canopy (5cm) with corresponding values of photon flux density (µmol m^{-2} s^{-1}); and (c) stomatal conductance to water vapours for 30cm and 5cm together. Error bars represent +/- 1.s.e.

4.4 Discussion

4.4.1 Limitations of Study

Unfortunately, this field study was affected by equipment failure. The nature of the study was such that it was governed by the weather conditions; reliable measurements using an IRGA require dry leaves, meaning that early morning dew caused a problem; in these cases measurements commenced in the later hours of the morning when the leaves had dried. The requirement for dry, sunny days with significant ozone levels and suitable conditions for measurements of gas exchange and to prevent water logging of equipment meant that when the sun came, which was infrequent in 2005, field work was spontaneous. Due to the lack of suitable periods in the spring/early summer period of 2005, there were only a few days of measurement available.

For these reasons, these data can only provide a brief and limited snapshot of conditions influencing ozone exposure and uptake in woodland and grassland canopies in the Yorkshire Dales.

4.4.2 Woodlands

The stomatal conductance of *V.riviana and M. perensis* (Fig 4.5 to 4.7) species remained low throughout the day, with values ranging between 20 and 80 mmol m^{-2} s^{-1}. This reflected the very low penetration of light into the woodland canopy; with the exception of short periods with sun flecks, the photon flux density remained below 100 μmol m^{-2} s^{-1}. Shade tolerant species are highly reactive to sun flecks and

can rapidly open and close stoma in response (Tinoco-Ojanguren and Pearcy, 1992). However, in this study, there was no evidence that short periods of increased photon flux density were associated with increased stomatal conductance.

These stomatal conductance values are somewhat lower than has been reported by other studies; for example, a study by Leuschner (2002) looking at stomatal responses of common European woodland species to varying Vapour Pressure Differences (VPD) recorded stomatal conductance within the range of 150 - 500 mmol m^{-2} s^{-1}.

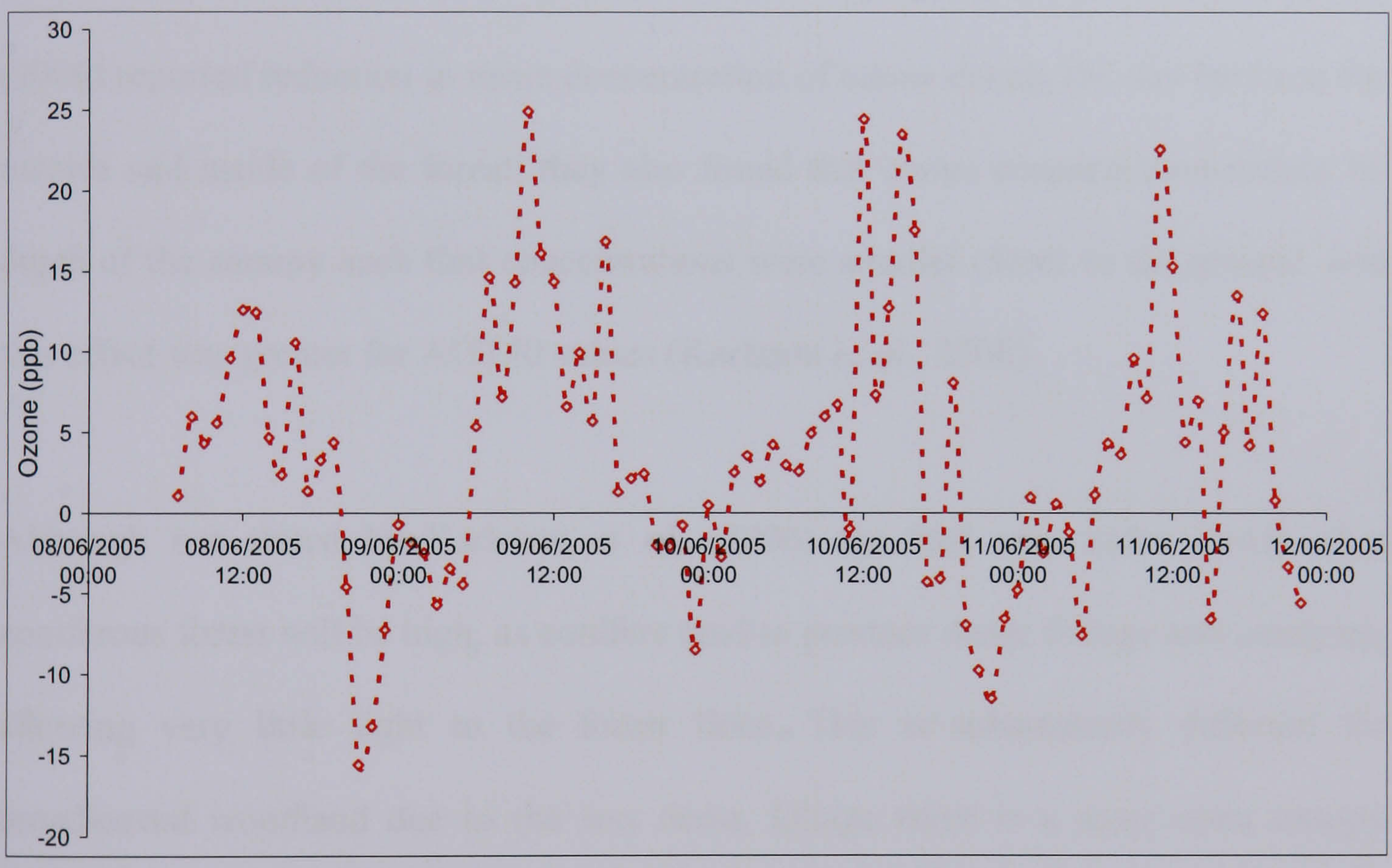


Fig 4.15
The difference in mean hourly ozone (ppb), expressed as outside-inside, recorded from 08/06/05 – 12/06/05 inside and outside of Grass Wood.

Fig 4.15 shows the difference in ozone concentration between the outside and the inside of the Grass Wood (outside-inside), recorded between 08th June and 12th June 2005. Ozone concentrations were reduced inside the woodland during the day; the

maximum difference between inside the woodland and outside was approx. 30ppb a reduction of 50%. A similar decrease of ozone concentration during the day was observed in the first study period. Ozone concentrations within the woodland rarely exceeded 40ppb over the study periods, even when concentrations were higher outside of the canopy, and hence the AOT40 index might be particularly reduced inside the woodland.

The only detailed study of ground-level ozone concentrations within a forest is the recent study of Karlsson *et al.* (2005) in a Norway spruce forest. Karlsson *et al.* (2006) reported reduction in mean concentration of ozone during the day between the outside and inside of the forest; they also found that ozone concentration reduce by depth of the canopy such that concentrations were smaller closer to the ground and this effect was greater for AOT40 values (Karlsson *et al.*, 2006).

Although not stated by Karlsson *et al*, (2006) the leaf area index (LAI) of a coniferous forest will be high, as conifers tend to produce dense foliage and canopies, allowing very little light to the forest floor. This is substantially different for broadleaved woodland due to the less dense foliage there is a more open canopy structure and thus more air movement. In broadleaved woodland it is possible that AOT40 values for this reason could be not dissimilar throughout the canopy.

Karlsson *et al.* (2005) reported a reduction in mean ozone concentrations of 10-40% at night-time in a spruce forest compared to levels outside the forest. At Grass Wood,

in contrast, the levels inside the woodland were consistently greater at night than outside, with a maximum difference in mean of 15ppb. The higher levels at night within Grass Wood in comparison to the study by Karlsson *et al*, (2005) may arise from the nature of a broadleaved woodland canopy being less dense than a coniferous one and thus allowing a relatively high degree of air mixing. In addition to this, the possibility exists that the tree canopy is inactive during the night and this allows a greater degree of ozone to penetrate the canopy over night.

4.4.3 Grasslands

The dense canopy of the grassland, at Ingleborough, reduces the ozone exposure of *C.palustris* leaves in the lower canopy. Jaggi *et al*, (2006) report a decline in mean ozone concentration in grassland as 36% between 0.90m and 0.25m. Fig 4.10 shows how ozone concentrations are reduced between 0.30m and 0.05m; the mean difference in hourly mean concentrations being 54% with much variation throughout the day; levels of reduction are not comparable to the study by Jaggi *et al*., (2006) possibly due to the much higher canopy in their study and the shorter more dense foliage present in the meadow at Colt Park Wood.

Finkelstein *et al*. (2004) reports the rate of decline of mean ozone concentration with depth into an herbaceous plant canopy, within a stand of *Rudbeckia laciniata,* they found reductions of 50% between 1m and 20 cm above ground level, which they related to LAI. These results are more comparable to the figures obtained from Colt Park Wood.

For leaves receiving very limited irradiance, the stomatal conductance of the leaves within the canopy is relatively high. It is possible that VPD, rather than irradiance, is controlling stomatal conductance of this species during the day, since in periods of high irradiance the conductance of the upper canopy leaves is reduced, while that of the lower canopy leaves increases (Figs 4.10 (c) & 4.13 (c)). This may be related to an increase in VPD which might be less marked within the canopy where conditions are presumably more stable. In terms of ozone concentrations this is indeed the case, with lower variation within the canopy than above it.

There are potentially significant contrasts between the time course of stomatal conductance in the upper canopy and recorded concentrations of ozone. As concentrations of ozone increase in the upper canopy after midday (Fig 4.10), there is a decrease in conductance of the upper leaves (Fig 4.11 (a)). The second period of study at Ingleborough (8-9 July 05), was a much hotter day and Fig 4.13 shows that the day had potential for high levels of ozone, early that morning levels were between 40-70ppb and had been high over night. Conductance was erratic, high in the morning and very low from midday onward. High levels of stomatal conductance, as seen in the upper canopy, are likely to lead to high flux of ozone; however when ozone peaks are likely, e.g. following midday and into the afternoon, there is reduced conductance in the most exposed leaves of *C.palustris*

It is very difficult to compare this study to the wider effect of ozone exposure on upland meadows as this study is limited to one uncharacteristic species *C.palustris*.

Comparison with data from the Newcastle University team (pers. Comm.) suggests *C.palustris* shows quite high night-time conductance levels; this is consistent with the relatively high conductance at 5cm despite much the lower irradiance, in this study. However, it does suggest that *C. palustris* could be unusual and therefore to gain a deeper understanding, more research is needed on other grass and forb species.

4.4.4. Conclusions

In Grass Wood, reductions in ozone concentrations were less than in *C.palustris* canopy at Colt Park Wood. However, these are still relatively low concentrations of ozone, under the assumed threshold of 40ppb for vegetation used in the AOT40 index. Hence, the risk of impacts based on AOT40 may be small.

Furthermore the impact of ozone on woodland ground flora may be further limited by the low values of stomatal conductance. However, measurements reported here were made late in the season for many woodland species when the tree leaf canopy was fully developed. Therefore, due to lower irradiance levels conductance may be low only in this season. It is possible that there may be less reduction of ozone and higher conductance earlier in the growing season.

For C.palustris, the data suggest high values of conductance are maintained within the canopy, but that ozone levels are much reduced; these would greatly reduce the potential impact on leaves and species at lower levels of the canopy. However, this needs to be supported by measurements within real mixed grassland canopies at different stages of development and before and after cutting or following grazing.

Overall this study shows the importance of assessing ozone exposure and conductance together within specific micro-habitats relevant to different species. However, this study provides only a snapshot and much more data is needed to make more definite conclusions.

5. *Chapter 5:* The effects of ozone exposure and its interaction with shade on woodland ground flora communities.

5.1 Introduction

Semi-natural British woodlands have been in decline over the last 150 years in response to changing land use and the intensification of agriculture. What little woodland is left intact is under pressure from climate change, pollution, especially nitrogen deposition, and lack of management.

Woodland ground flora is a very sensitive community and in Britain has evolved alongside hundreds of years of woodland management by man. This has created a woodland flora adapted to a system of cyclical change and regeneration depending heavily on a seed bank for the latter. A study by Kirby *et al* (2005) suggests that over the last 30 years the result of external negative drivers on our woodlands has resulted in an overall shift towards more shaded assemblages of woodland ground flora, and a loss of open habitat spaces within woodlands as they become more closed and less management takes place. Upland broadleaved woodland are a scarce habitat type and with ambient levels of ozone predicted to increase, especially in upland areas, these communities are likely to be impacted by this air pollutant. There has been much research looking at the effect of ozone pollution on individual tree species or forest composition (see review by Karnosky *et al.* (2006)) but very little focussing on the impacts of ozone on ground vegetation.

The results from Chapter 2 suggested that species of woodland habitats may be relatively sensitive to ozone, while Chapter 3 showed that characteristic woodland spring bulb species may also be sensitive to ozone. Chapter 4 demonstrated that, at least in a relatively open upland wood such as Grass Wood, the ozone concentrations experienced by ground flora species may be lower yet still reflective of the concentrations outside.

Thus there is evidence from the work reported in previous chapters of this thesis that ozone may be a significant threat to the ground flora of upland woods of high conservation value. However, the effects of ozone need to be considered at a community level, as the competition between species may significantly affect the impacts of ozone (e.g. Barbo *et al.*, 1998; etc). The only study that has examined the effects of ozone on woodland ground flora was undertaken by Barbo *et al.* (1998), who exposed early successional communities after woodland clearance to elevated ozone in open-top chambers, found that ozone exposures caused shifts in the competitive interactions between plants and leading to alteration in the community structure. In addition, Barbo *et al.* (1998) found higher species richness, diversity, and evenness in their control treatments.

However, no studies have been conducted of effects of ozone on the emergence and development of species that occurs regularly in spring in deciduous woodlands. The aim of the study reported in this chapter was to examine the response of communities

emerging in mesocosms using soil taken from an upland broadleaved woodland in order to: i) assess the effects of ozone on community composition and biodiversity and ii) to investigate if these effects are altered by the introduction and persistence of shade. The study was based on material taken from the Grass Wood site including mesocosms established from different areas under different management regimes.

5.2 Methods

5.2.1 Introduction

The intention of the experiment was to use soil collected from upland woodland to create small mesocosms, which would grow from the propagules already present within the soil. This would create a more realistic mixture of species within the individual mesocosms than would occur if species were planted or sown. This approach meant that the effects of ozone developed from the earliest stages of seedling emergence. It also meant that species that some species which are difficult to grow from seed, such as *Mercuralis perrenis*, could be present in this study grown from root stock present. Three different experiments were undertaken.

5.2.2 Soil Collection

Table 5.1 gives details, for three experiments, of soil origin from within Grass Wood, date of collection, fumigation length and the type of treatments applied. Soil was collected from Grass Wood (SD985655) in the autumn or spring. A site description for Grass Wood can be found in Chapter 2.

Experiment 1, was an initial exploratory experiment and soil was predominantly collected from an area of wet woodland adjacent to an open ride on the far side of the wood. To get a good representation of all the species within the wood, soil was also taken from various other adjacent rides for this experiment. For Experiments 2 and 3, all soil taken was confined to a specific 2m x 2m area within a ride in an attempt to reduce the variability between individual mesocosms that was seen in Experiment 1.

Table 5.1
Experimental parameters.

Site	Collection Site	Soil Collected	Exposure Dates	Exposure Length (days)	Number of mesocosms	Treatments	Species Richness
1	Site 1 – Open wet area of woodland	Oct 04	09/11/2004 -22/02/2005	98	19	O_3+ Shade CFA+ Shade	40
2	Site 2- Closed 7-8 year old Coppice plot	Mar 05	27/03/2005 – 26/08/2005	149	39	O_3+ Light O_3+ Shade CFA + Light CFA + Shade	14
3	Site 1	Apr 05	02/05/2005 - 08/09/2005	105	38	O_3+ Light O_3+ Shade CFA + Light CFA + Shade	30

Original in colour

The surface of the soil was brushed clean of any leaf litter and debris and dug out with a spade to a maximum depth of 50cm. Soil was collected in large bags, which were sealed and transported back to Bradford University Dept of Geography and Environmental Science. Here soil was mixed thoroughly, all large root components were removed from the soil and the soil was then transferred to planters.

Shallow tray style planters were used for Experiment 1, as shown in Fig 5.1 (a). These mesocosms were excellent for short-term germination due to the larger exposure of the soil surface; however later in the experiment the roots started to grow through the bottom of the trays. These trays were 23cm x 17cm, with a depth of 7cm, giving a total surface area of 391cm^2.

For Experiments 2 and 3 a deeper, round standard plant pots were used; these prevented root growth through the bottom during the length of the experiment. These mesocosms are shown in Fig 5.1 (b). These round pots were 16cm in diameter and 14cm deep, making the surface area 200cm^2.

(a)

(b)

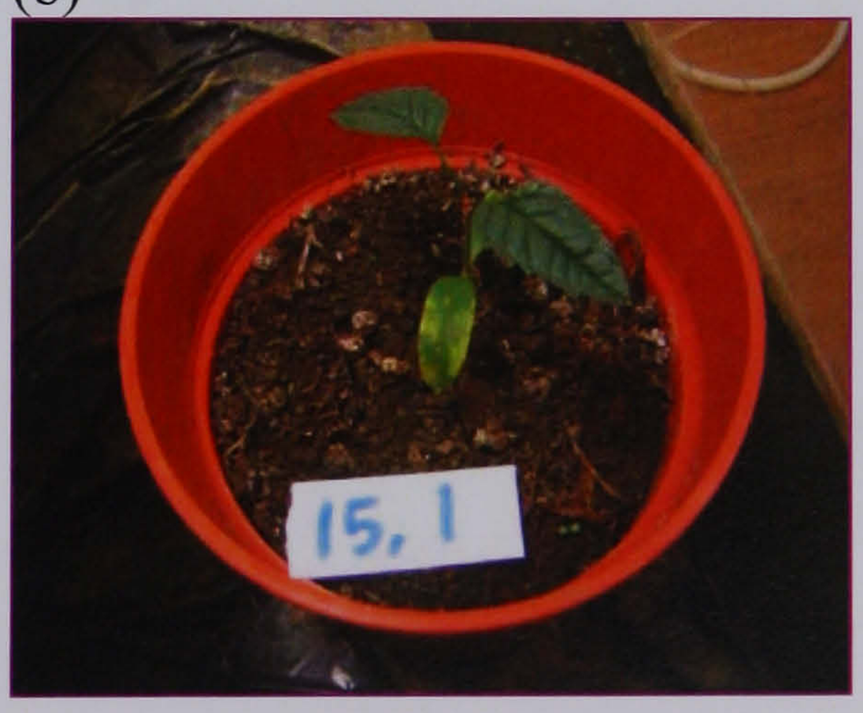


Fig 5.1
(a) Tray used for experiment 1 and (b) Pots used in experiment 2 and 3

5.2.3 Experimental Design

The three experiments were run consecutively with overlap between Experiments 2 and 3. Table 5.1 gives details of experimental parameters including exposure dates and species richness for Experiments 1, 2 and 3.

Mesocosms were assigned an identification number and were randomly distributed between the 8 Perspex growth chambers (details of fumigation chambers are given in Chapter 2 Section 2.4) fed with either charcoal filtered air (CFA) or a target concentration of 80ppb of ozone. Monitoring of ozone concentrations was as described in Chapter 2.

Until the first seedlings appeared, there was no ozone added to the system. For Experiment 1 this was one week, and for Experiments 2 and 3 this was two weeks prior to the start of ozone treatment.

5.2.3.1 Light and Shade Treatments

Experiment 1 had four weeks of full light in the chambers (PAR at plant height: 90 $\mu mol\ m^{-2}\ s^{-1}$) to simulate the early spring period before the canopy starts to grow. The mesocosms were then covered with a muslin shade which reduced the photon flux density by one third, to 66 $\mu mol\ m^{-2}\ s^{-1}$. Following a further four week interval, a further muslin shade was added, reducing the to 30 $\mu mol\ m^{-2}\ s^{-1}$.

The same shading regime applied to Experiments 2 & 3; the only difference was that these experiments had mesocosms that were in the full light treatment for the entire fumigation period. Each chamber had a light and a shade treatment in Experiments 2 & 3.

Light levels recorded in Stour Wood, Essex, show that ground flora in mature coppice (over 7 years old) received in midsummer, less than 1% of the total direct radiation (Mason & Macdonald; 2002). In midsummer, at midday, in sunny conditions, light levels are typically around 2000 μmol m^{-2} s^{-1}; light levels fluctuate depending on weather condititions so a good estimate of average light levels in summer will be around 1000 μmol m^{-2} s^{-1} over a 16-hour sunny day. The highest light levels in the internal chambers would be equivilant to 10% of this figure and is similar to light levels recorded at Grass Wood, in Chapter 4. Reducing light levels further would reflect the deeper shade exhibited by the further development of a canopy throughout the growing season.

5.2.3.2 Measuring growth

At the start, and throughout the fumigation period, on a weekly basis when possible, species richness, diversity and cover were measured. Also at weekly intervals, the number and cover of each species in each individual mesocosm was recorded.

Species identification was based on personal prior knowledge of identification of woodland seedlings and plants. Immediately after seedling emergence, species

identification was often problematical. Therefore, individuals were marked and followed until a definitive identification was possible. Percentage cover was estimated by eye for each mesocosm per species. From these data, species richness (r) and diversity indices (Shannon diversity index (H') and Pielou index of equitability (J)) were calculated (Pielou, 1975). Shannon diversity index is given by the equation:

$$H' = -\sum_{i-1}^{S} p_i \ln p_i$$

where:

S: The number of species (species richness (r))

n_i: The number of individuals in each species; the abundance of each species.

N: The total number of all individuals.

P_i: The relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community: n_i/N.

Pielou index of equitability (J) is given by the equation:

$$J = \frac{H'}{\ln(S)}$$

The Shannon diversity index (H') is a commonly used index in ecological studies. The advantage of this index over others, e.g. Simpsons diversity Index (D), is that it takes into account the number of species and the evenness of spread of these species. The index is increased either by having additional unique species, or by having a greater species evenness. Alternatively, a community which is dominated by one species will have low diversity with this index.

Peilou index of equitability (J) is a common companion of the Shannon diversity index. Equitability indices describe the spread of species within a community; high

equitability shows that all species are present in similar abundance levels; whereas low equitability indicates dominance of one or a few species.

At the end of the fumigation period the above ground biomass was removed, separated into species and used to calculate dry biomass.

5.2.4 Statistical Analysis

Statistical analysis was carried out in SPSS (v14, SPSS Inc.). The species that emerged from individual mesocosms were extremely variable, and therefore formal statistical analysis of treatment effects on individual species was not possible, due to the large number of missing values. For this reason, species were grouped into Ellenberg light categories, to allow for a more robust analysis of whether ozone affected the balance between species adapted to different woodland micro-habitats.

All analysis was done on the basis of individual mesocosms and collated into treatment groups (Table 5.1). Chambers were arranged in banks of 2 spread across four bays (blocks); to alleviate any effect of positioning of chambers each block had an O_3 and a CFA chamber and all data were analysed for between block effects. The significance of treatment effects for the data for all parameters were then tested using a one-way, two-way or three-way analysis of variance, as appropriate. The assumptions of ANOVA were tested and met using SPSS (version 14). As in Chapter 2, significance of $p < 0.10$ was accepted as significant due to the highly variable nature of the material in this study. Where data was recorded over the exposure

period a repeated measure analysis was used to highlight changes over time for continuous sets of data.

Where there is significance block effects are recorded in the text. In experiments 2 and 3, light*gas, light*block and light*gas*block effects were also tested; due to the introduction of a light treatment.

5.3 Results

Results are presented for each experiment in turn, in the order in which they were performed. The results from these experiments are then compared in the Discussion section.

5.3.1 Experiment 1

5.3.1.1 Introduction

Experiment 1 had two treatments: CFA + Shade (control) and O_3 + Shade (ozone). Table 5.2 lists all the species which occurred on at least one occasion, ranked by order of shade tolerance according to the Ellenberg Index for Light (L) (Hill *et al*; 2004), with percentage frequency values (i.e. the percentage of mesocosms in which they were found) of species present at the end of the experiment.

Many species occurred in a small proportion of mesocosms and only a small number of species were present in high frequencies. However, there are more species present in the ozone treatments overall than in the control; this is especially notable with species from Ellenberg group 7, i.e. species of open/light habitats. Many more of these species occurred only in the ozone treatments and those that were present in both treatments were often in higher frequencies in ozone than the control. In particular, *S. procumbens* was present in 82% of ozone mesocosms but only 50% in the control. 14 species listed in Table 5.1 were only present in the ozone treatment whereas only 3 occurred only in the control treatments.

Original in colour

Table 5.2

Species Present in Order of Shade Tolerance according to the Ellenberg Light Index (L) and Percentage Frequency (Fq) Values for the end of the Experiment.

NB. Percentage frequency indicates the % of mesocosms in which the species is present

Ellenburg Index (L)		Species	% Fq		
Description	Rank		Ozone	Control	Total
Shade	**3**	*Mercuralis perrenis*	9	-	5
		Urtica doica	9	10	10
Between 3 and 5	**4**	*Oxalis acetosella*	36	30	33
		Veronica montana	9	-	5
Semi-shade	**5**	*Hyacinthoides non-scripta*	9	10	10
		Luzula pillosa	9	-	5
		Lysimachia nummularia	45	10	29
		Potentilla sterelis	27	50	38
		Scrophularia nodosa	18	20	19
		Silene doica	18	-	10
Between 5 and 7	**6**	*Chamerion agustifolium*	9	-	5
		Deschampsia caespitosa	36	30	33
		Epilobium hirstutum	9	-	5
		Epilobium montanum	-	10	5
		Fragaria vesca	9	-	5
		Glechoma hederacea	18	10	14
		Holcus mollis	-	20	10
		Ranunculus repens	27	30	29
		Rubus fructicosus agg	73	60	67
		Valeriania officinalis	18	20	19
		Veronica chamaerodys	27	20	24
		Viola riviania	45	50	48
Light and partial shade	**7**	*Agrostis tenuis*	27	10	19
		Alchemilla glabra	9	-	4
		Cerastium fontanum	27	-	14
		Fillipendula spp.	9	-	5
		Holcus lanatus	55	50	52
		Hypericum humifusum	64	60	62
		Juncus spp.	-	10	5
		Luzula campestris	36	20	29
		Plantago lanceolata	27	-	14
		Poa annua	9	10	10
		Prunella vulgaris	36	10	24
		Rumex acetosella	9	-	5
		Rumex obtusifolium	9	-	5
		Sagina procumbens	82	50	67
		Stellaria media	18	20	19
		Trifolium repens	9	-	5
Light Loving	**8**	*Cardamine hirstuta*	73	60	67

Original in colour

5.3.1.2 Species Richness and Abundance

Table 5.3 gives the results of the repeated measures analysis for species richness (r) and abundance. Fig 5.2 gives the mean values for (a) r and (b) abundance, over time (90 days). There is a significant effect of time, showing an increase in species richness (r) over time ($P<0.01$) and abundance ($P<0.05$) although this is much less clear for abundance.

Values of r are fairly similar in the two treatments at the start of the experiment. As time progresses, the ozone treatment has a slightly higher mean than the control treatment. There is a slight decrease in r in the ozone treatment following the introduction of the second shade while there is no change in the control treatment in relation to the change in light.

Table 5.3
Results of a repeated measures analysis for species richness (r) and abundance (A); **(a)** within subject and **(b)** between subject effect
* = P<0.10; **= P<0.05, ***=P<0.01

(a)	d.f	F	
		r	A
Time	7	40.33 ***	3.21 **
Time * Block	21	1.08	1.03
Time * Gas	7	0.59	0.63
Time *Block *Gas	21	0.74	0.53

(b)	d.f.	F	
		r	A
Block	3	2.68	3.22*
Gas	1	4.18*	3.91*
Block * Gas	3	0.12	0.16

There is a small effect of gas treatment overall ($P<0.10$) relating to a greater mean species richness in the ozone treatment than in the control. There are no significant interactions with time for species richness.

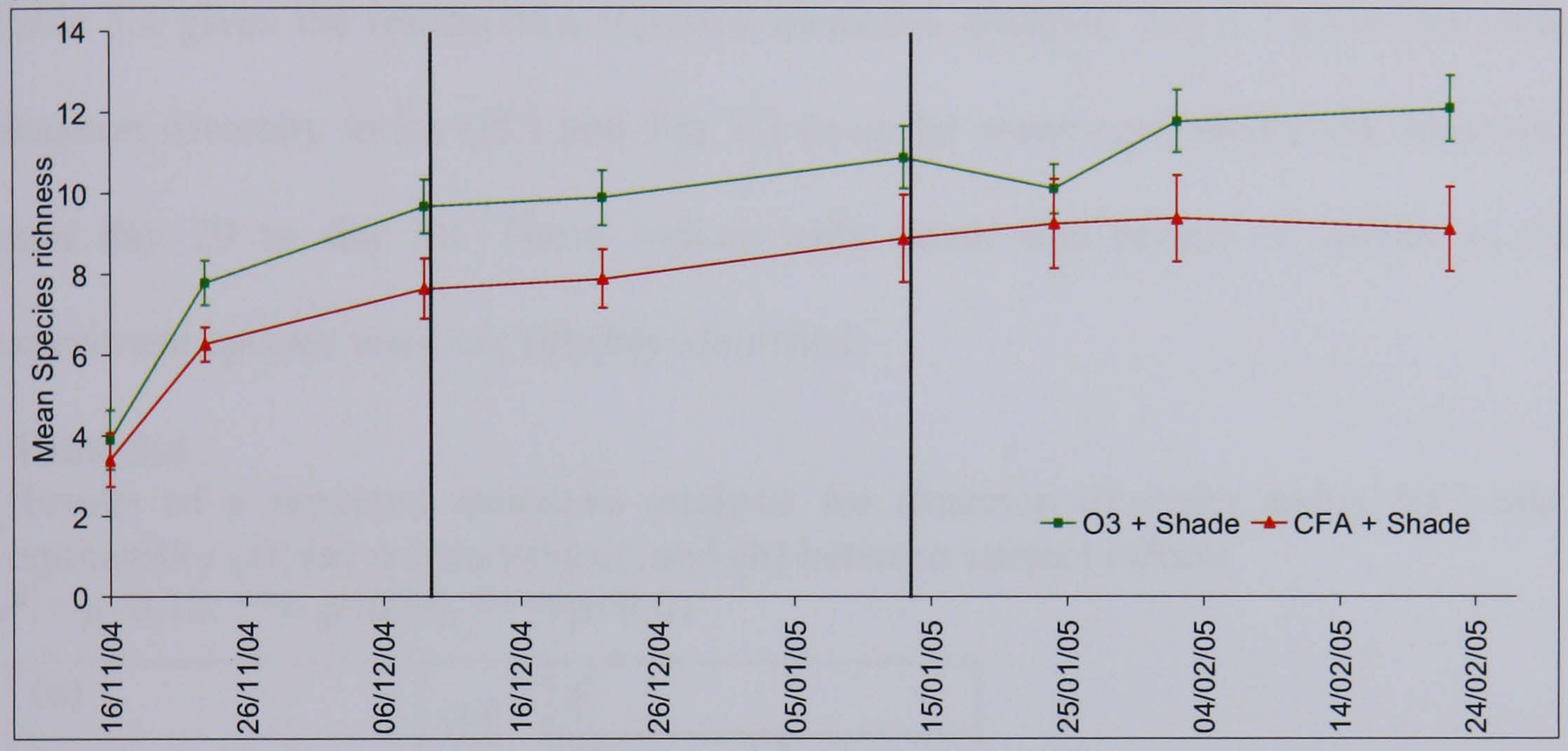


Fig 5.2
Mean **(a)** Species Richness (r) and **(b)** Abundance: The first marker denotes 33% reduction in light; the second marker denotes 66% reduction: Error bars represent +/- 1s.e.

Mean abundance is higher in the ozone treatment than in the control from the start, and there is little change over time. There is a small effect of gas treatment ($P<0.10$).

As this difference was observed at the start of the experiment, this is not an effect of treatment but rather a chance difference in initial abundance. There is also a block effect (P<0.10).

5.3.1.3 Shannon diversity index (H') and Pielou index of equitability (J)

Table 5.4 gives the results of a repeated measures analysis; Fig 5.3 gives the mean Shannon diversity index (H') and Fig 3.3 (a-c) the mean equitability (J), measured from day 29 to day 90. These indices only cover this period as earlier in the experiment species were not reliably identified.

Table 5.4
Results of a repeated measures analysis for Shannon diversity index (H') and equitability (J); **(a)** within subject, and **(b)** between subject effects.
* = p<0.10; **= p<0.05, ***=p<0.01

(a)	d.f.	F	
		H'	J
Time	5	3.36 **	1.78
Time * Block	15	3.17 **	3.95 ***
Time * Gas	5	2.74 **	2.60 **
Time *Block *Gas	15	1.58	2.42 **

(b)	d.f.	F	
		H'	J
Block	3	2.60	2.37
Gas	1	0.16	1.92
Block *Gas	3	1.30	3.33 *

There is a statistically significant effect of time for H' (P<0.05) but not for J. Diversity is initially higher in the ozone treatment but following the second shade there is a drop in the mean ozone H' and at the same point increase in control mean H'. In addition there is a significant gas/time interaction on the value of H' (P<0.05).

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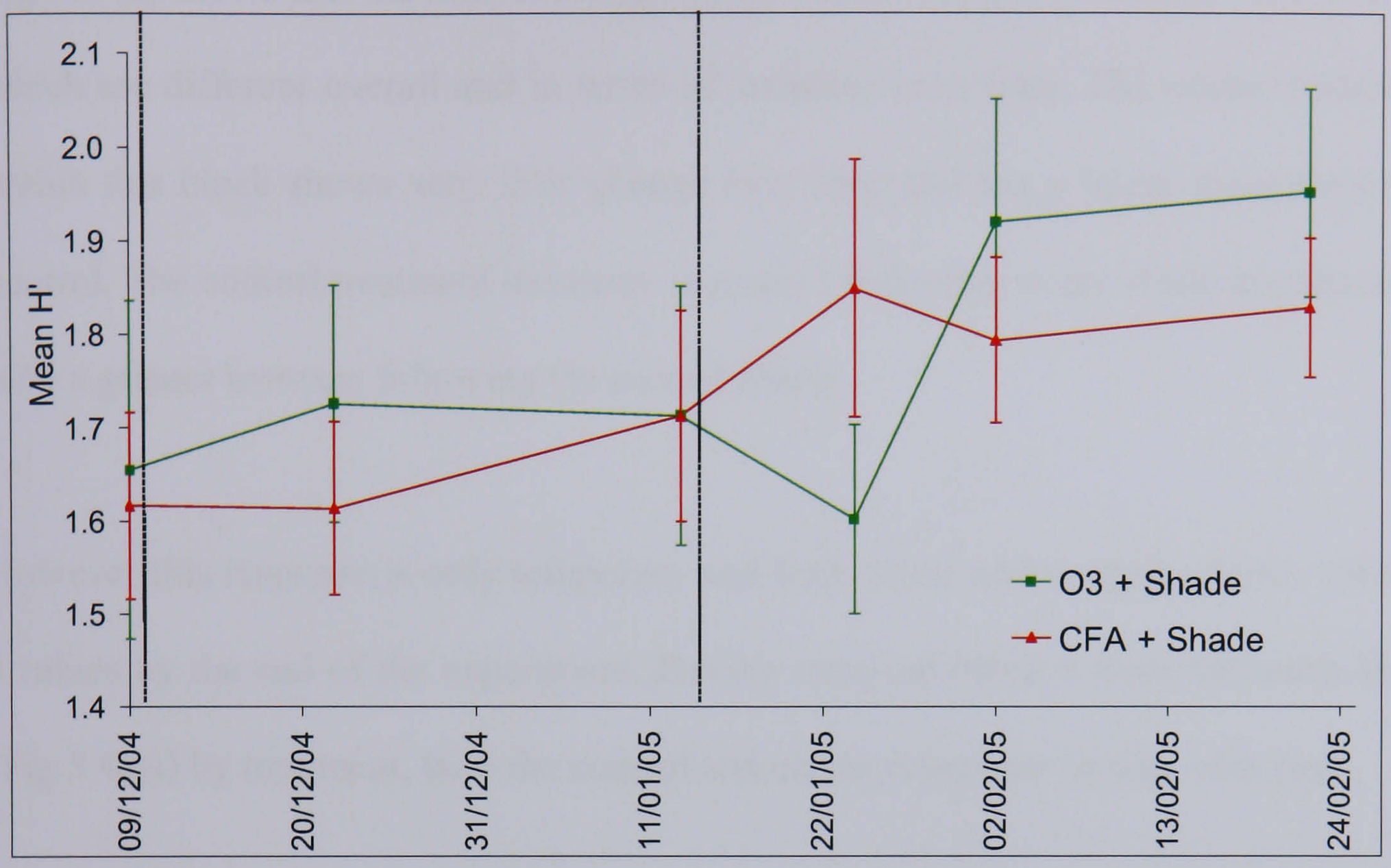

Fig 5.3
Mean H' (+/- 1 s.e.) from day 29-90, the first marker denotes 33% reduction in light; the second marker denotes 66% reduction

There is only one point of change over time for J. After the introduction of the second shade, there is a small increase in the value of the control and a small decrease for the ozone. There is a significant interaction of time/gas ($P<0.05$) confirming that the treatment effect varied significantly over time.

Block Effects

For J, there is a significant time/block interaction ($P<0.01$), time/block/gas interaction ($P<0.05$), and an overall block/gas effect ($P<0.10$). Fig 5.4 (b) shows the block/time effect, showing that the most different block, in terms of variation over time, is Block 4. However a Tukey HSD test indicates that Block 1 and 2 are most different from each other ($P<0.10$) although there is no significant overall block effect.

Fig 5.4 (c) shows that the time/block/gas effect relates directly to changes in Block 4, which are different overall and in terms of variation over time. The ozone treatment within this block shows very little change over time and has a lower mean than the control. The control treatment increases in mean J following every shade introduction with a greater increase following the second shade.

However this response is only temporary and both ozone and control achieve similar J values by the end of the experiment. Having removed block 4 from the mean for J (Fig 5.4(c)) by treatment, both the control and ozone values are similar over time.

Table 5.5 gives the repeated measures analysis for J, having excluded block 4; there is no longer any significant gas effects and the biggest effects are now the difference in reaction over time of Blocks 1 and 2 ($P<0.10$).

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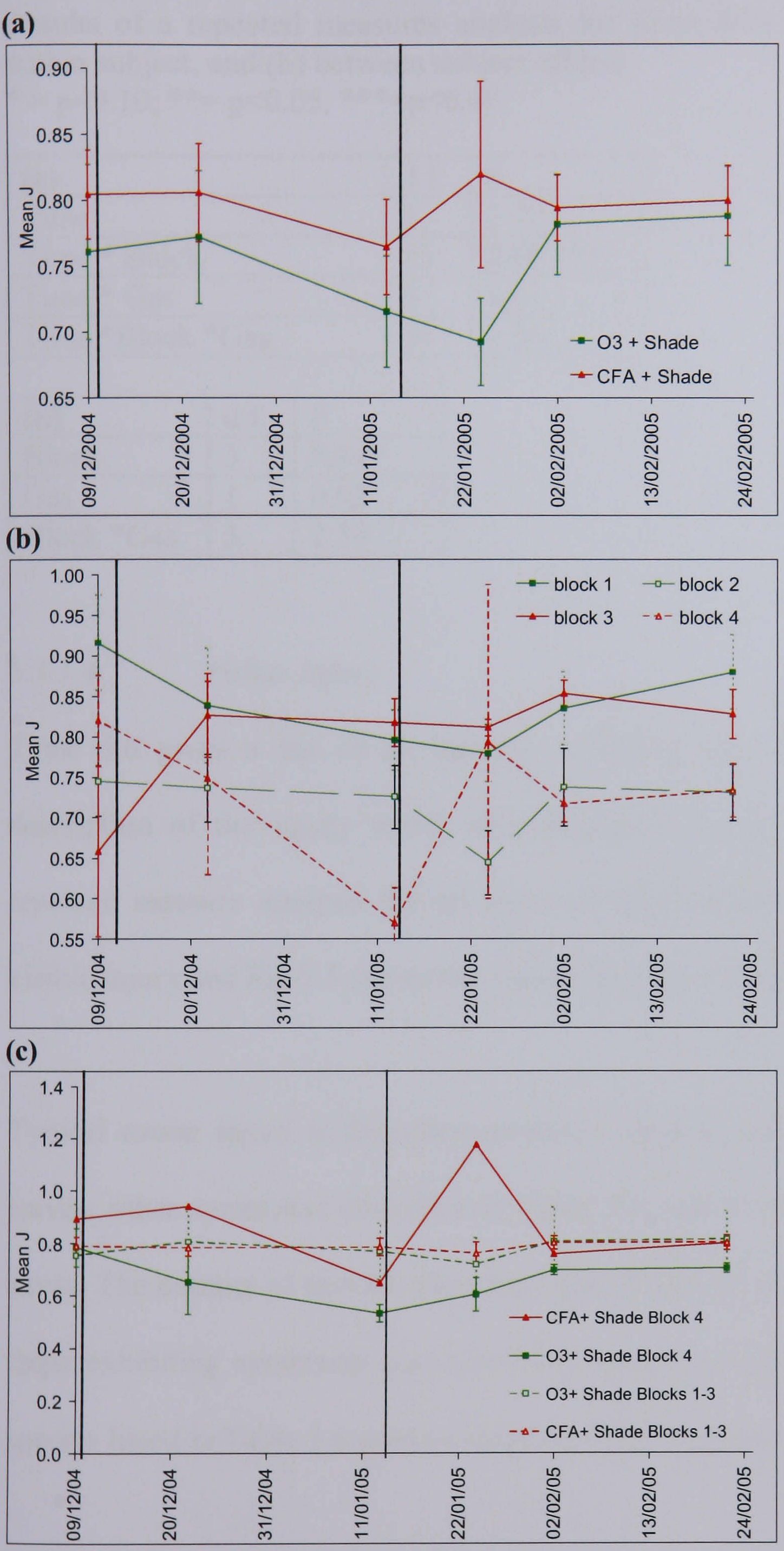


Fig 5.4

(a) Mean J **(b)** Mean J by block and **(c)** Mean J by treatment for Block 4 and 1-3 combined (+/- 1.s.e.). The first marker denotes 33% reduction in light; the second marker denotes 66% reduction

Table 5.5
Results of a repeated measures analysis for Equitability (J) excluding block 4; **(a)** within subject, and **(b)** between subject effects.
* = p<0.10; **= p<0.05, ***=p<0.01

(a)	d.f.	F
Time	5	1.72
Time * Block	15	**2.89** ***
Time * Gas	5	0.61
Time *Block *Gas	15	1.74

(b)	d.f.	F
Block	3	**3.54***
Gas	1	0.02
Block *Gas	3	2.30

5.3.1.4 Foliar Injury

Table 5.6 gives a list of all species exhibiting any type of foliar injury and a description of the injury which they displayed. Table 5.7 gives the results of a repeated measure analysis for all types of foliar injury recorded as percentage of visible injury and Fig 5.5 shows the mean injury over time for both treatments.

Typical ozone injury is described as plants showing flecking or stippling on their leaves; other symptoms described in Table 5.6 tend to be generally associated with stress. The number of species showing signs of typical ozone injury was smaller than those exhibiting symptoms associated with stress such as chlorosis. Eight of the 29 species listed in Table 5.6 showed these typical symptoms.

The most seriously affected species in terms of visible injury were *G.hederacea*, which showed heavy flecking over most of its leaves in all cases in the ozone treatment. *H.humifusim* was another species showing severe injury to the leaves; it

was affected in all mesocosms in the ozone treatments, and showed both stress and typical ozone symptoms.

Ozone injury first occurred after the first week of fumigation; however this was only a small amount. Fig 5.5 shows that the first few weeks there was little foliar injury, but then a steep increase in the ozone treatment occurred. There is a general increase over time, with small depressions following the introduction of each shade. There is a significant effect of time ($P<0.01$), there is also a significant interaction of time with gas treatment ($P<0.01$) and an overall highly significant effect of ozone treatment ($P<0.01$).

Table 5.6
Foliar injury observed through exposure period

Species	Stipple	Fleck	Chlorosis	Premature senescence	Reddening
	Many small red, purple or other pigmented spots	Many small yellow spots	Areas of leaf with a bright yellow colour	Leaf drop	areas or deep red or purple
Agrostis spp.				x	
Alchemilla glabra			x		
Cardamine hirstuta			x		
Deschampsia ceaspitosa				x	
Fillipendula spp.			x		
Fragaria vesca				x	
Glechoma hederacea		x			
Holcus linatus	x		x	x	
Hypericum humifusum	x	x	x		x
Luzula campestris			x		
Luzula pillosa			x		
Lysimachia nummularia	x				x
Mercuralis perrenis			x		
Oxalis acetosella			x		
Plantago lanceolata	x		x		
Poa annua			x		
Potentilla sterelis			x		
Prunella vulgaris				x	
Ranunculus repens			x		
Rubus spp	x	x	x		
Rumex acetosella			x		
Rumex obtusifolium			x		
Scrophularia nodosa		x			
Stellaria media				x	
Trifolium repens			x		
Valeriania officinalis			x		
Veronica chamearodys		x			
Viola riviania			x		

Table 5.7
Results of a repeated measures analysis for Percentage Foliar Injury **(a)** within subject, and **(b)** between subject effects.
* = p<0.10; **= p<0.05, ***=p<0.01

(a)	d.f.	F
Time	4.29	**6.02*** **
Time * Block	12.86	1.23
Time * Gas	4.29	**4.01*** **
Time * Block * Gas	12.86	0.91

(b)	d.f.	F
Block	3	0.19
Gas	1	**23.61** ***
Block * Gas	3	0.04

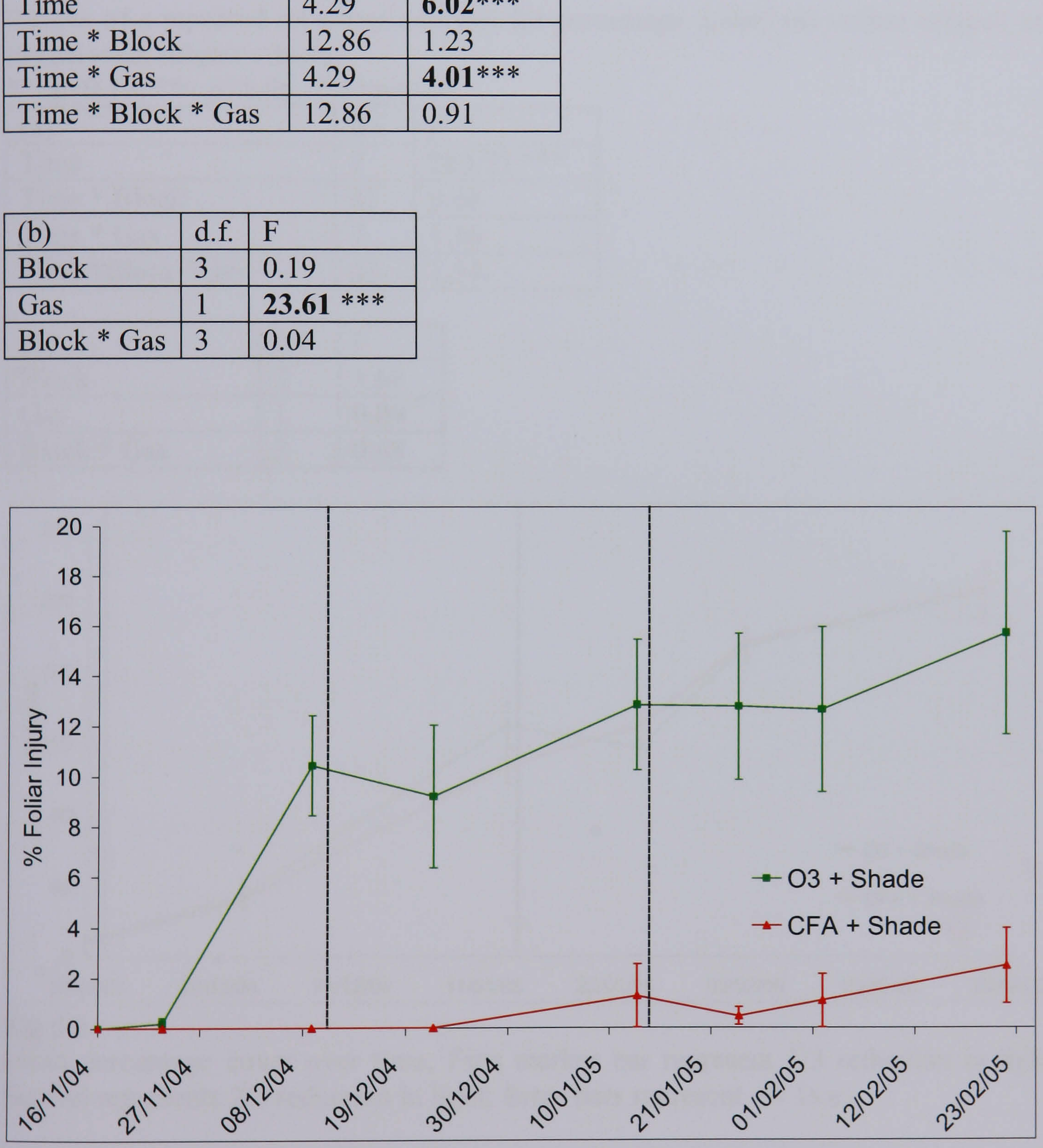


Fig 5.5
Mean Percentage Foliar Injury; the first marker denotes 33% reduction in light; the second marker denotes 66% reduction; Error bars represent +/-1s.e.

5.3.1.5 Percentage Cover

Table 5.8 gives the results of a repeated measures analysis for percentage cover and Fig 5.6 shows the changes in cover over time for each treatment. The two treatments showed almost identical patterns of cover growth over the experiment.

Table 5.8
Results of a repeated measures analysis for percentage cover; **(a)** within subject, and **(b)** between subject effects:
* = p<0.10; **= p<0.05, ***=p<0.01

(a)	d.f.	F
Time	7	**203.77** ***
Time * Block	21	0.68
Time * Gas	7	1.16
Time *Block *Gas	21	1.54

(b)	d.f.	F
Block	3	1.66
Gas	1	0.09
Block * Gas	3	0.68

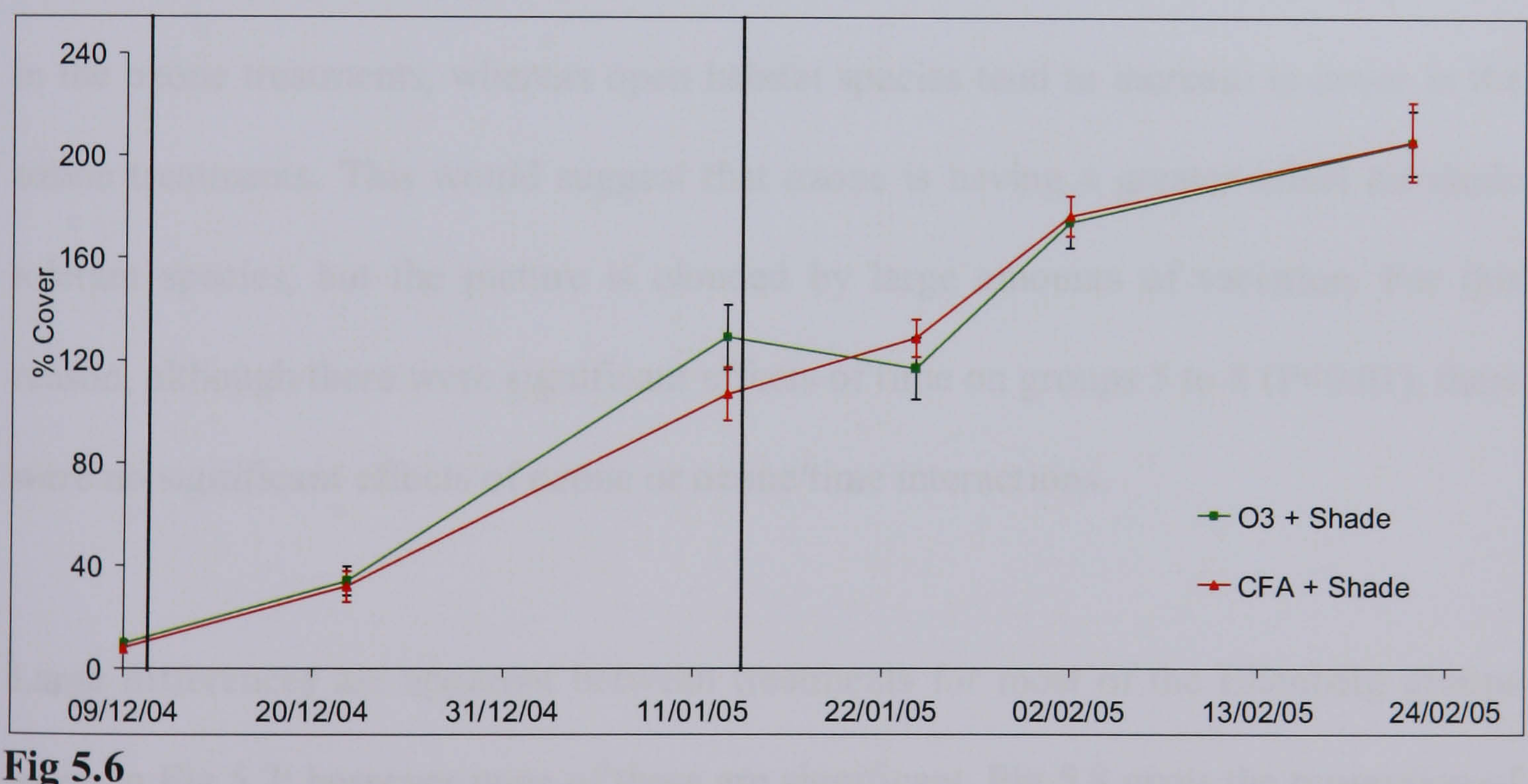


Fig 5.6
Mean percentage cover over time; First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light; Error bars represent +/- 1s.e.

Following the second reduction in light there was a small reduction in mean percentage cover of the ozone treatment but it quickly recovers. There was a significant effect of time on percentage cover ($P<0.01$); no other effects on mean percentage cover were found to be significant.

5.3.1.6 Cover by Ellenberg Light Index

The species present in the mesocosms were ranked in terms of their sensitivity to shade (Table 5.2) using the Ellenberg Index for light (Hill *et al.*, 2004). The majority of species came from groups 6 and 7. Fig 5.7 shows the changes over time (from 29-90 days) of the mean percentage cover for these Ellenberg groups by treatment. Results from repeated measures analysis are given in Table 5.9.

In general, Fig 5.7 shows that species with tolerance to shade tend to decrease in cover in the ozone treatments, whereas open habitat species tend to increase in cover in the ozone treatments. This would suggest that ozone is having a greater effect on shade tolerant species, but the picture is clouded by large amounts of variation. For this reason, although there were significant effects of time on groups 5 to 8 ($P<0.01$), there were no significant effects of ozone or ozone/time interactions.

Large differences are apparent between treatments for most of the Ellenberg groups given in Fig 5.7; however none of these are significant. Fig 5.8 gives the proportion of the total cover represented by groups (a) 3-5, (b) 6 and (c) 7 & 8. This gives aid to understanding the different dynamics between treatments in the mesocosms for these.

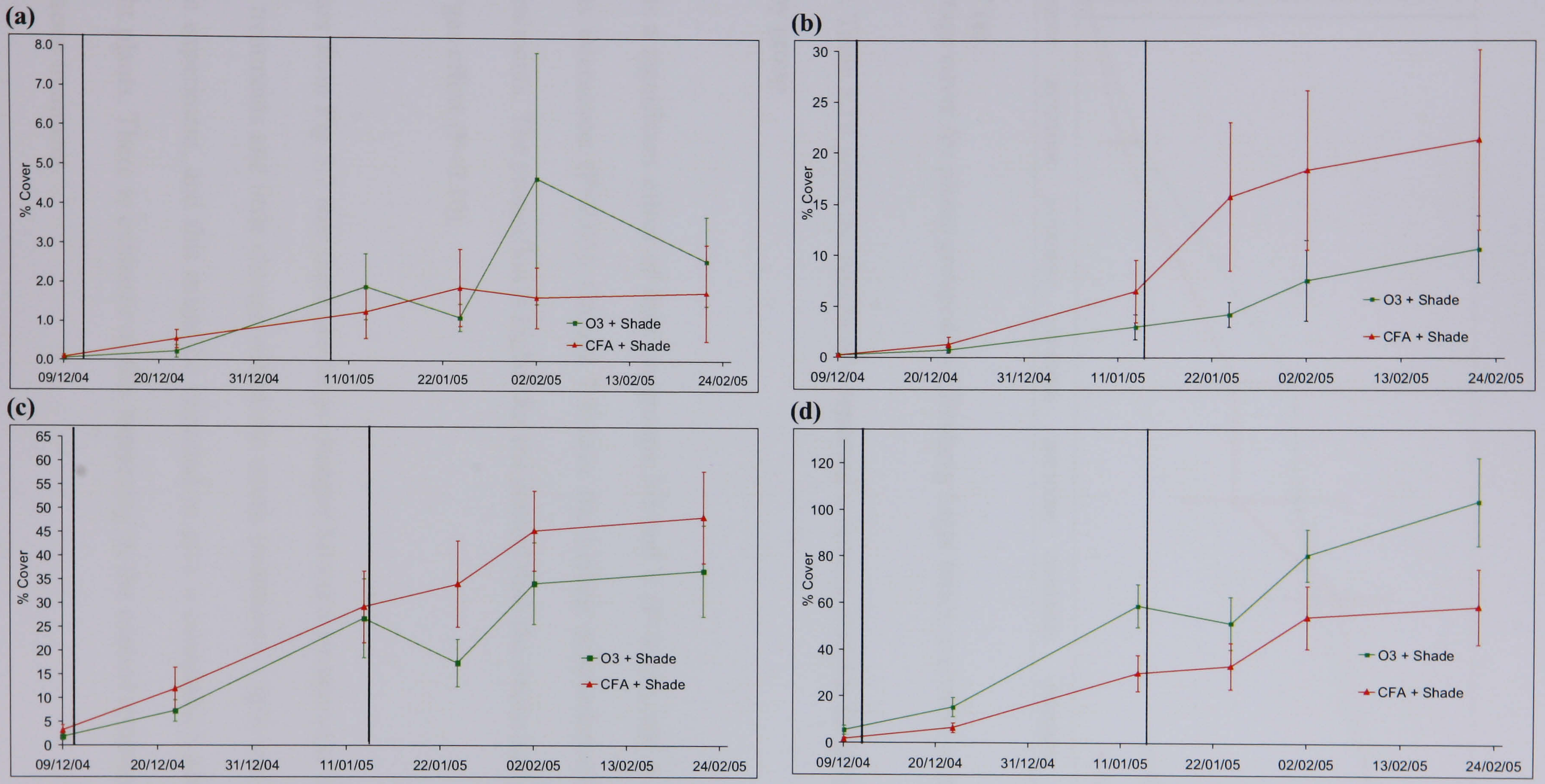


Fig 5.7
Mean percentage cover for plants grouped by Ellenberg Light Index over time; a) L3&4, b) L5, c) L6, d) L7, e)L8; First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light; Error bars represent +/- 1s.e.

(e)

Fig 5.7 (e)
Percentage cover for plants grouped by Ellenberg Light Index over time cont.

groups. Table 5.10 gives the results of a repeated measures analysis for proportion of cover by group

There is a significant effect of time on groups 3-5 and 6 ($P<0.01$), and a significant time/gas interaction ($P<0.05$). Groups 7-8 have the highest proportion of cover for both treatments. The proportion is higher for the control than the ozone and there is an overall gas effect ($P<0.10$).

It appears from Fig 5.7 that there are large changes between groups over time in the control treatments and little change within the ozone treatments. Light was decreased over the experiment, and this might be expected to give a competitive advantage to low light plants. There is evidence of this happening in the control treatment, but not in the ozone treatment.

Table 5.9
Results of a repeated measures analysis for cover ordered by Ellenberg Index for light; **(a)** within subject, and **(b)** between subject effects.
* = p<0.10; **= p<0.05, *** = P<0.01

(a)	3 & 4		5		6		7		8	
	d.f	F	d.f.	F	d.f.	F	d.f.	F	d.f.	F
Time	3.34	1.96	2.68	**10.71** ***	3.00	**25.30*****	2.93	**27.40** ***	5.00	**29.66** ***
Time * Block	10.03	1.00	7.94	0.77	8.98	1.03	8.79	1.00	15.00	0.48
Time * Gas	3.34	0.81	2.65	2.22	3.00	0.99	2.98	0.26	5.00	0.71
Time *Block *Gas	10.03	0.89	7.94	1.21	8.98	0.85	8.79	0.80	15.00	0.52

(b)	d.f.	3 & 4	5	6	7	8
Block	3	1.54	0.83	1.18	0.40	0.31
Gas	1	0.24	2.24	0.61	2.14	0.01
Block * Gas	3	0.08	1.02	0.18	0.61	0.05

Table 5.10
Results of a repeated measure analysis for proportion of cover ordered by Ellenberg Index for light; **(a)** within subject, and **(b)** between subject effects.
* = p<0.10; **= p<0.05, *** = P<0.01

(a)	3-5		6		7-8	
	d.f.	F	d.f.	F	d.f.	F
Time	5	**5.15*** **	4.35	**3.06** **	5	0.80
Time * Block	15	0.83	13.05	0.58	15	1.45
Time * Gas	5	**3.03** **	4.35	**2.94** **	5	1.06
Time * Block * Gas	15	1.64	13.05	**1.77***	15	0.80

(b)	d.f.	3-5	6	7-8
Block	3	0.60	1.02	0.91
Gas	1	1.78	2.32	**4.82***
Block * Gas	3	0.99	0.62	0.85

Original in colour

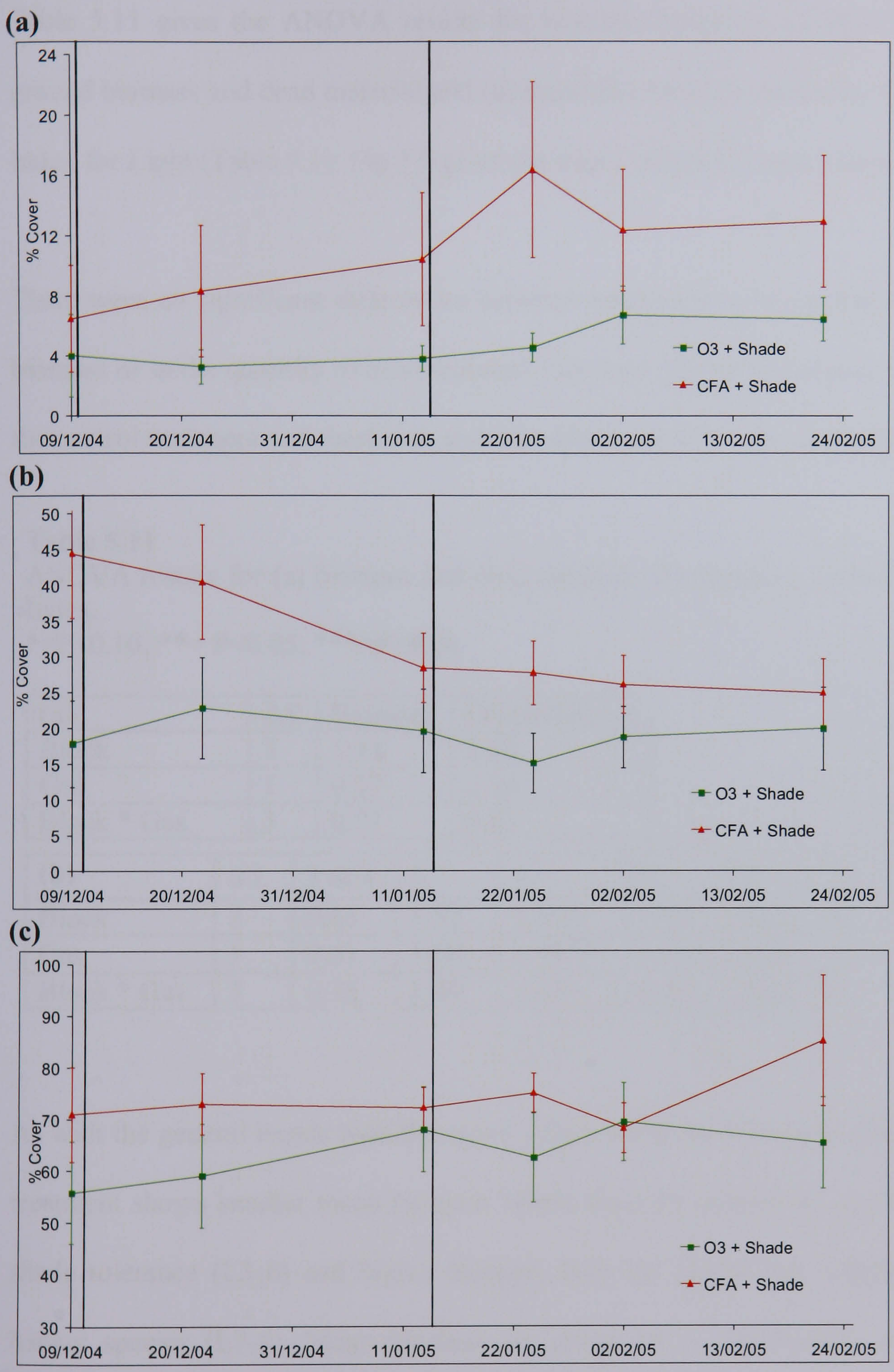


Fig 5.8

Mean percentage of cover for plants grouped by Ellenberg Light Index over time; **a)** L3-5, **b)** L6, **c)** L7-8; First marker bar represent 1/3 reduction in light, Second 2/3 reduction in light; Error bars represent +/1 s.e.

5.3.1.6 Biomass

Table 5.11 gives the ANOVA results for biomass based on (a) mean live above-ground biomass and dead material and (b) mean live biomass ranked by the Ellenberg Index for Light (Table 5.1). Fig 5.9 gives the mean values for each treatment.

There were no significant differences between treatments in mean live above-ground biomass or in the quantity of dead material. However the mean biomass was higher in the control treatment and there was also less dead material in the control treatment.

Table 5.11
ANOVA results for (a) biomass and dead material, (b) biomass, ranked by Ellenberg Index.
*=P<0.10, **= P<0.05, ***=P<0.01

(a)	d.f.	Biomass	Dead Material
Block	3	1.44	2.84
Gas	1	0.37	2.14
Block * Gas	3	0.51	0.21

(b)	d.f.	3 & 4	5	6	7	8
Block	3	0.63	1.52	3.04*	0.92	0.67
Gas	1	0.61	1.69	6.48**	0.25	0.01
Block * Gas	3	0.38	1.44	1.45	0.33	0.09

As with the general trends with the cover values using the Ellenberg index, the ozone treatment shows smaller mean biomass values than the control for species with high shade tolerance (L3-6) and higher biomass than the control for ruderals and open-habitat species (L7-8). Mean biomass for group L6 is significantly greater in the control treatment ($P<0.05$) than the ozone treatment.

Original in colour

Fig 5.9
Mean live above-ground biomass and dead material

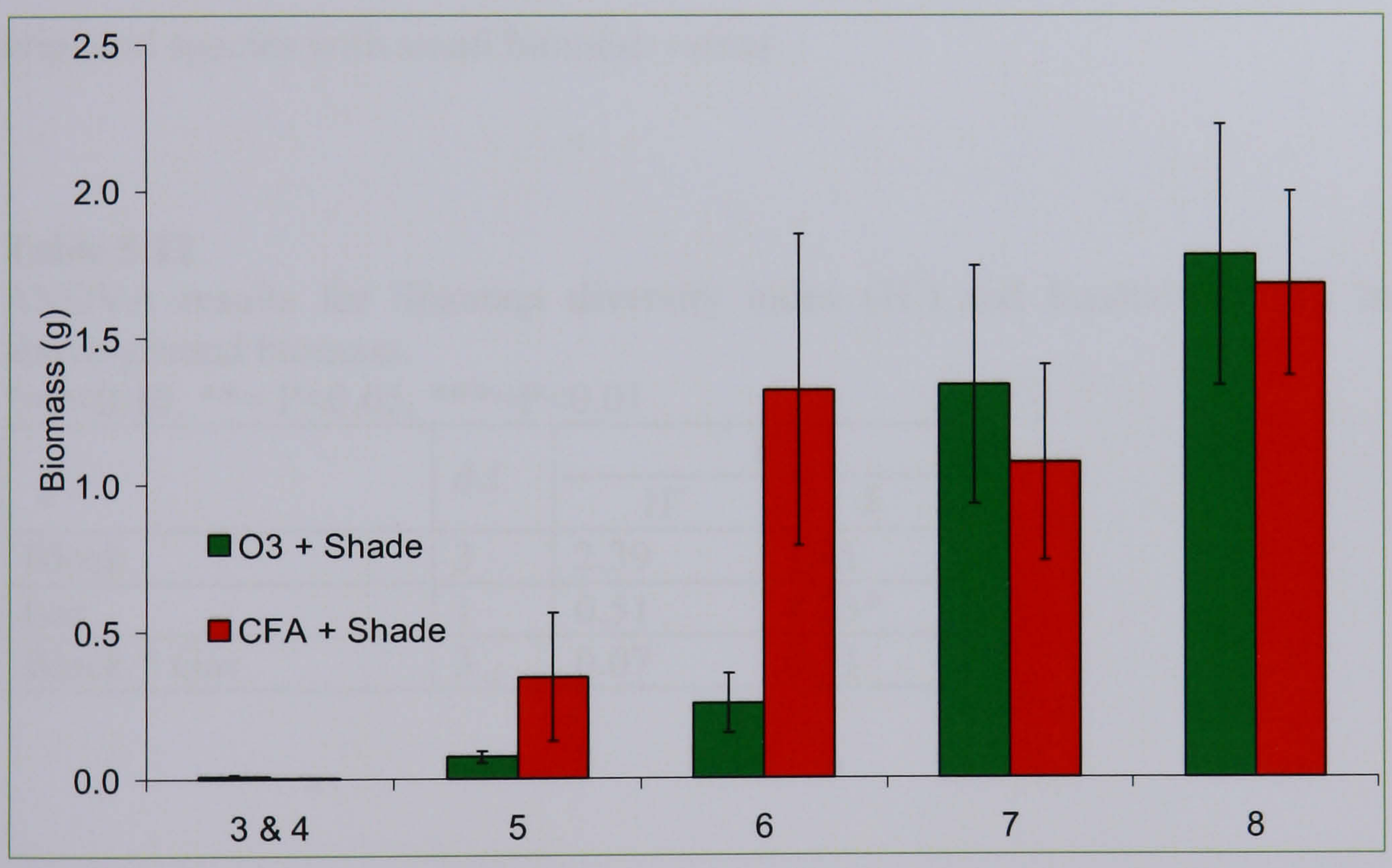


Fig 5.10
Mean live above-ground biomass in control and ozone treatments for different Ellenberg indices.

5.3.1.7 Shannon diversity Index – Biomass

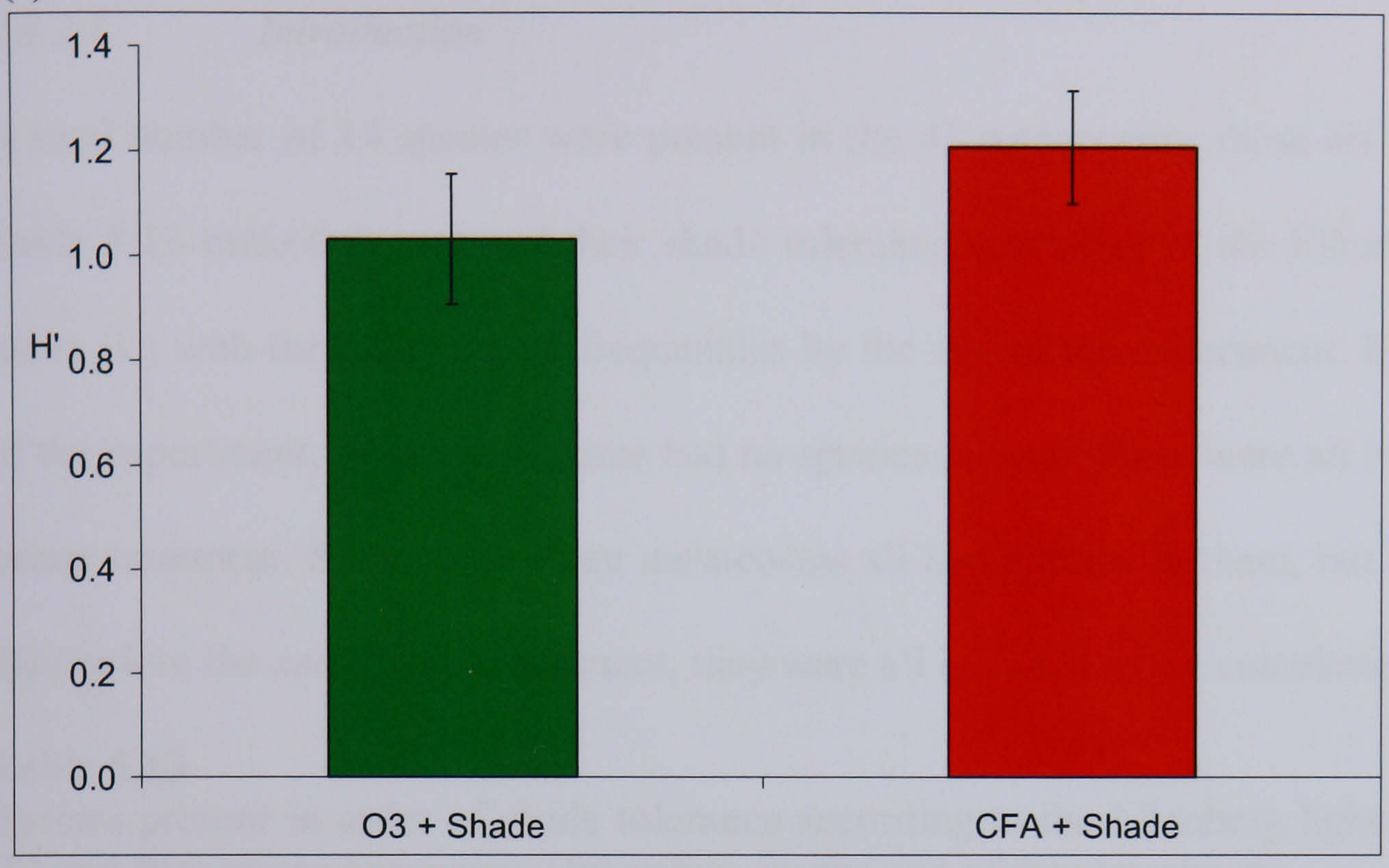
Table 5.12 gives the results of an ANOVA for diversity and equitability calculated on the basis of final live above-ground biomass. Fig 5.11 shows the mean diversity and equitability calculated from biomass for both treatments.

There was a small gas effect on equitability (P<0.10); the control treatment had a significantly higher mean equitability. Both H' and J were greater in the control treatment, although for H' this was not significant. These results suggest that the ozone treatment had a tendency to be dominated by one species and have a few peripheral species with small biomass values.

Table 5.12
ANOVA results for Shannon diversity index (H') and Equitability (J), based on above-ground biomass.
*=P<0.10, **= P<0.05, ***=P<0.01

	d.f.	F	
		H'	J
Block	3	2.39	1.81
Gas	1	0.51	4.23*
Block * Gas	3	0.07	0.71

(a)



(b)

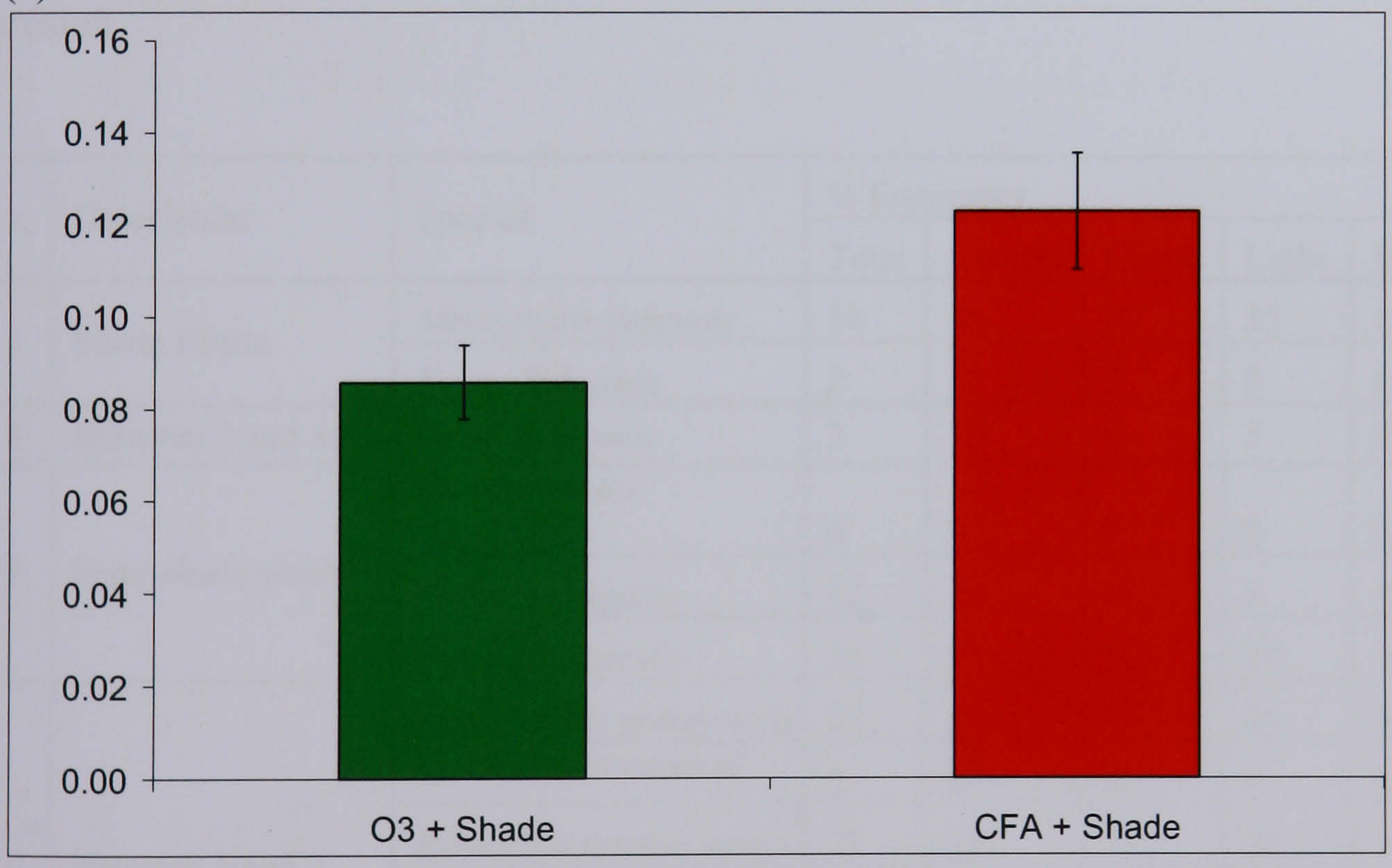


Fig 5.11
Mean **(a)** Shannon diversity index (H') and **(b)** Equitability (J) based on biomass values; Error bars represent +/- 1s.e.

5.3.2 Experiment 2

5.3.2.1 Introduction

A total number of 14 species were present in the 41 mesocosms; these are shown in Table 5.13 ranked in order of their shade tolerance according to the Ellenberg light index (L) with their percentage frequencies by the end of the experiment. By the end of the experiment, three mesocosms had no species present; these were all in the light ozone treatment. Since these three mesocosms all had species in them, but these had died before the end of the experiment, they were all included in the calculations.

Table 5.13
Species present in order of shade tolerance according to the Ellenberg light index (L) and Percentage Frequency (fq) values for the end of the experiment.
NB. Percentage frequency indicates the % of mesocosms in which the species is present

L	Description	Species	% Frequency				
			Total	Control	Ozone	Light	Shade
3	Shade Plants	*Mercurialis perennis*	34	40	29	55	14
		Fagus Sylvatica	2	0	5	0	5
4	Between 3 and 5	*Geum urbanum*	5	10	0	5	5
5	Semi-shade plants	*Hyacinthoides non-scripta*	0	0	0	0	0
		Primula vulgaris	12	10	14	5	19
		Potentilla sterelis	10	0	19	10	10
6	Between 5 and 7	*Aegopodium podagraria*	29	45	14	20	38
		Epilobium montanum	0	0	0	0	0
		Rubus fructicosus (agg.)	27	35	19	35	19
		Viola riviana	46	60	33	40	52
		Glechoma hederacea	2	0	5	0	5
		Fragaria vesca	20	20	19	10	29
7	Light and partial shade plants	*Luzula campestris*	7	5	10	5	10
		Hypericum humifusum	59	65	52	50	67

5.3.2.2 Species Richness and Abundance

Table 5.14 gives the results of a repeated measures analysis for both species richness and abundance. Fig 5.12 (a) shows the mean species richness (r) and Fig 5.12 (b) shows the mean abundance for all four treatments, over time. In Fig 5.12, the first marker on the graph indicates the start of the shade treatment and the second indicates the second shade increase applied to the shade treatments

There is a significant effect of time for both r ($P<0.01$) and for abundance ($P<0.01$). Both parameters showed an initial decrease then tended to decline slowly over the rest of the experiment. For r, gas treatment is significant overall ($P<0.05$) and also shows an interaction with time ($P<0.10$). Fig 5.12 (a) shows that the control treatments in both shade and light have a higher mean than that of the ozone treatments overall, but this difference was not apparent at the start of the experiment. There was no significant effect of light overall or interacting with time on the value of r, nor were there any significant interactions between light and ozone treatments.

Abundance values were greater in the control treatment and show a significant overall effect on gas treatment ($P<0.05$), but in contrast to effects on species richness, there was no significant gas/time interaction: Fig 5.12 (b) shows the effect was apparent from the start of the experiment. There was also an overall effect of light treatment ($P<0.10$). Fig 5.12 (b) shows the shade treatments having a higher mean than the light treatments, but this effect was also apparent from the start of the experiment.

There is an overall block/light interaction with abundance (P<0.10) and a significant block/gas/light interaction (P<0.05).

Table 5.14
Results of a repeated measures analysis for Species Richness (r) and Abundance (A), **(a)** within subject and **(b)** between subject effects;
* p<0.10, ** p<0.05, ***p<0.01

(a)	r		A	
	d.f.	F	d.f.	F
Time	4.28	**17.83*** **	3.60	**10.56*** **
Time*Gas	4.28	**2.02***	3.60	0.30
Time*Light	4.28	0.99	3.60	0.45
Time*Gas*Light	4.28	1.60	3.60	0.73
Time*Block	12.84	0.76	10.81	1.21
Time*Block*Gas	12.84	1.16	10.81	1.23
Time*block*Light	12.84	1.46	10.81	1.02
Time*Block*Gas*Light	1.58	0.97	10.81	0.75

(b)	d.f.	F	
		r	A
Block	3	0.47	0.99
Gas	1	**6.39****	**6.66****
Light	1	0.55	3.48*
Block*Gas	3	1.79	2.26
Block*light	3	0.89	**2.37***
Gas*Light	1	0.14	0.10
Block*Gas*Light	3	1.41	**3.43****

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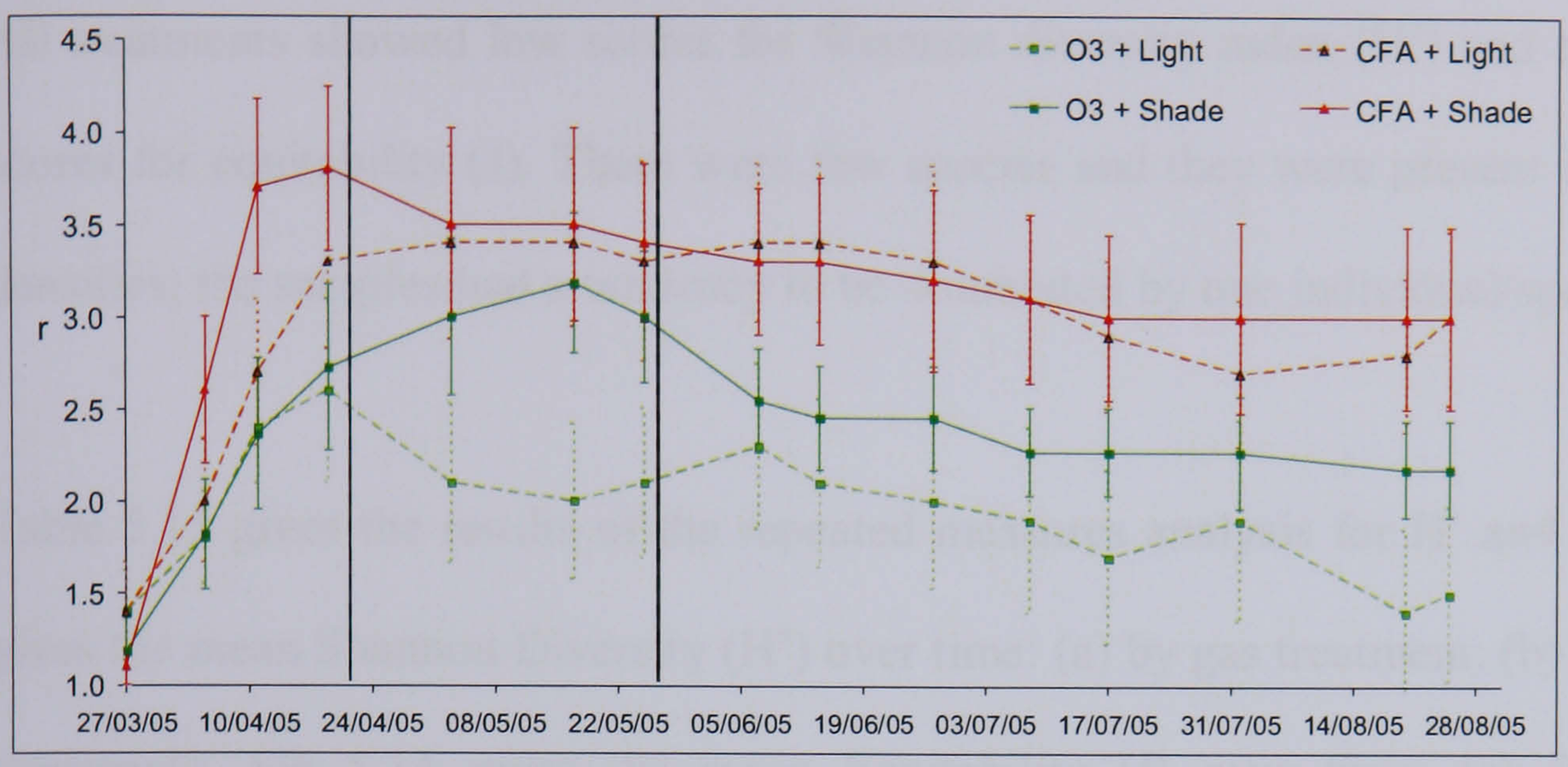


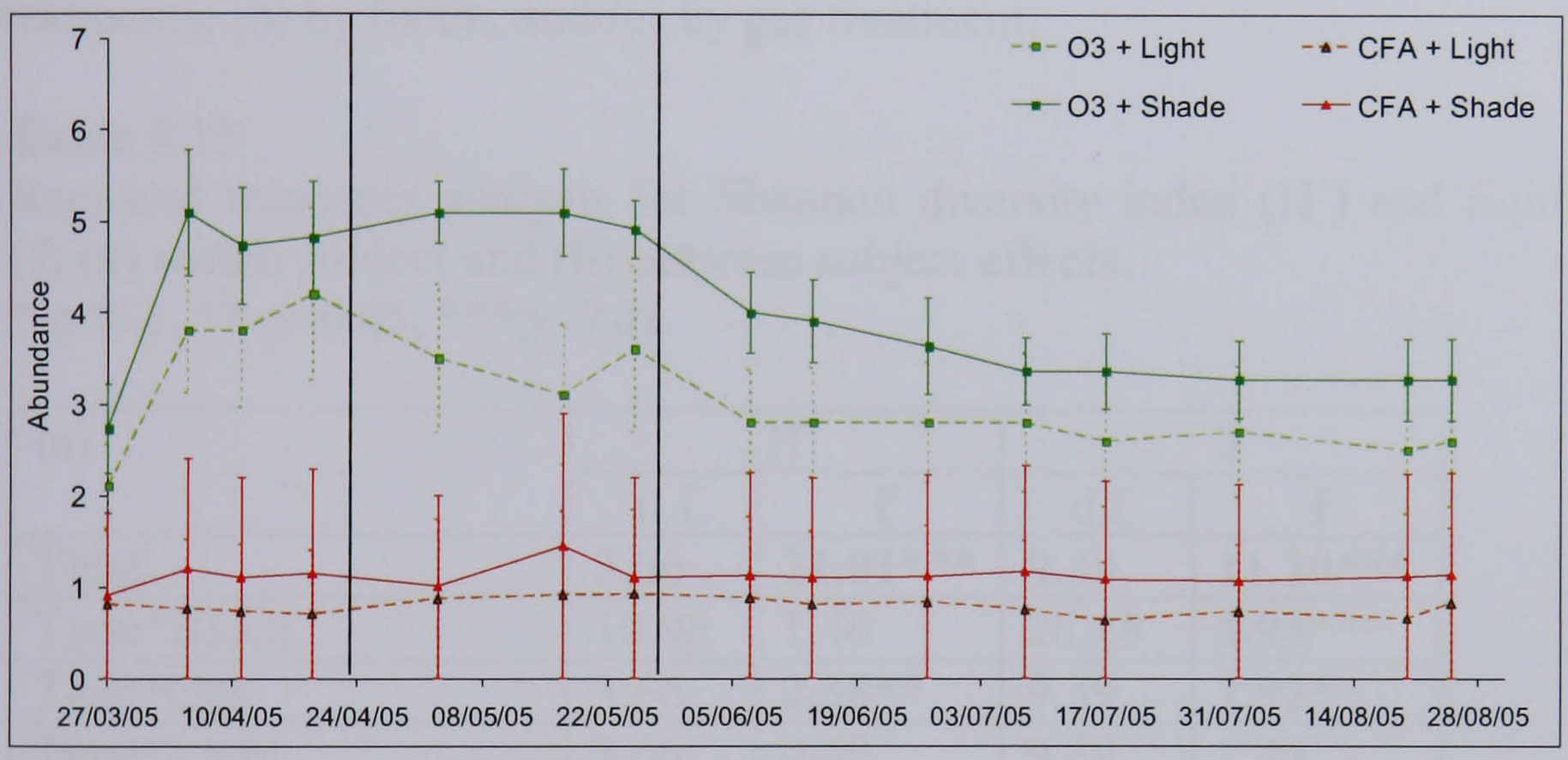


Fig 5.12
Mean **(a)** Species Richness (r), and **(b)** Abundance
First marker bar represent 1/3 reduction in light; Second represents 2/3 reduction in light.

Original in colour

5.3.2.3 Shannon diversity index (H') and Equitability (J)

All treatments showed low scores for Shannon diversity index (H') and equally low scores for equitability (J). There were few species and they were present in very low densities; the samples had a tendency to be dominated by one individual/species.

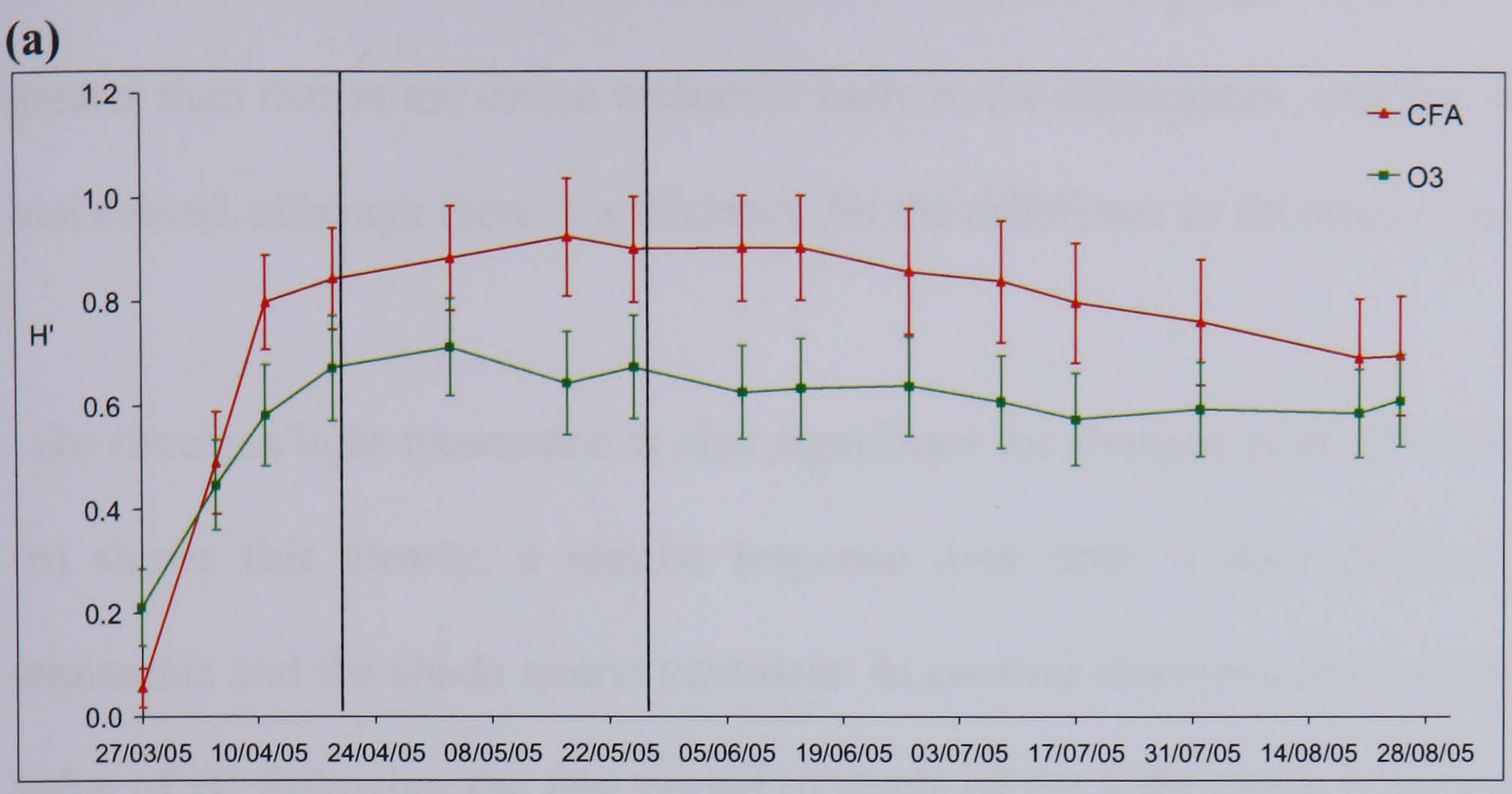
Table 5.15 gives the results of the repeated measures analysis for H' and J. Fig 5.13 gives the mean Shannon Diversity (H') over time: (a) by gas treatment; (b) for all four treatments. Fig 5.14 gives the mean Equitability (J) over time: (a) for all four treatments; (b) by block; and (c) by gas treatment.

Table 5.15
Repeated measures analysis for Shannon diversity index (H') and Equitability (J) **(a)** within subject and **(b)** between subject effects.
* p<0.1, ** p<0.05, ***p<0.01

(a)	H'		J	
	d.f.	F	d.f.	F
Time	3.66	**21.01*** **	9.39	**11.20*** **
Time*Block	10.98	1.46	28.18	**2.94*** **
Time*Gas	3.66	**2.58** *	9.39	**1.92** *
Time*Light	3.66	0.49	9.39	1.49
Time*Block*Gas	10.98	0.90	28.18	1.36
Time*block*Light	10.98	1.07	28.18	**1.80*** **
Time*Gas*Light	3.66	**2.84** *	9.39	**4.02*** **
Time*Block*Gas*Light	10.98	0.27	28.18	**1.48** *

(b)	d.f.	F-values	
		H'	J
Block	3	0.27	0.24
Gas	1	2.20	0.11
Light	1	0.43	0.45
Block*Gas	3	1.70	1.55
Block*light	3	0.99	1.48
Gas*Light	1	0.00	0.07
Block*Gas*Light	3	0.69	0.23

Original in colour



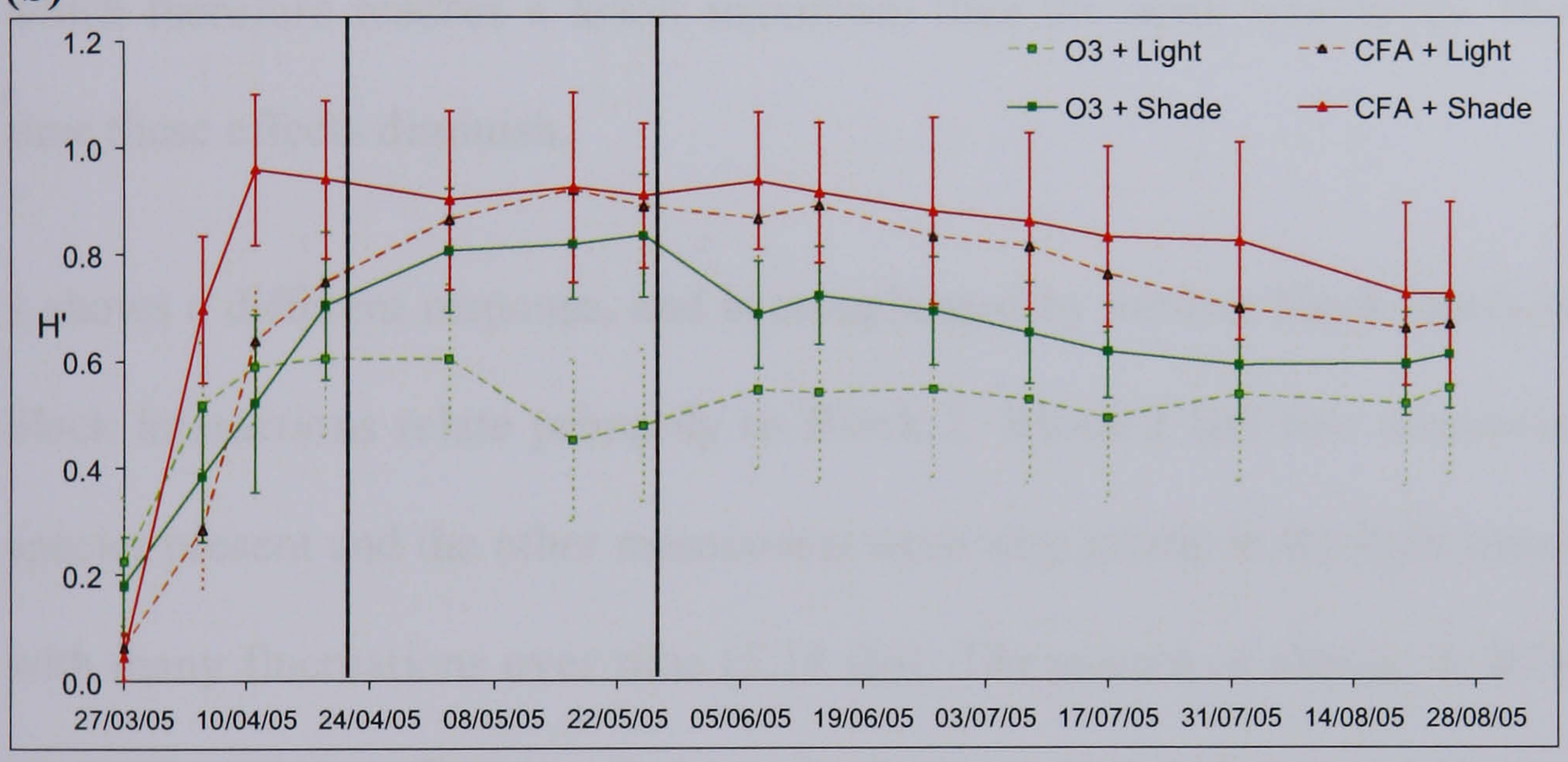


Fig 5.13
Mean Shannon diversity index (H'): **(a)** averaged over gas treatment; and **(b)** Shannon diversity index for all four treatments, +/- 1.s.e.
First marker bar represent 1/3 reduction in light, second represents 2/3 reduction in light.

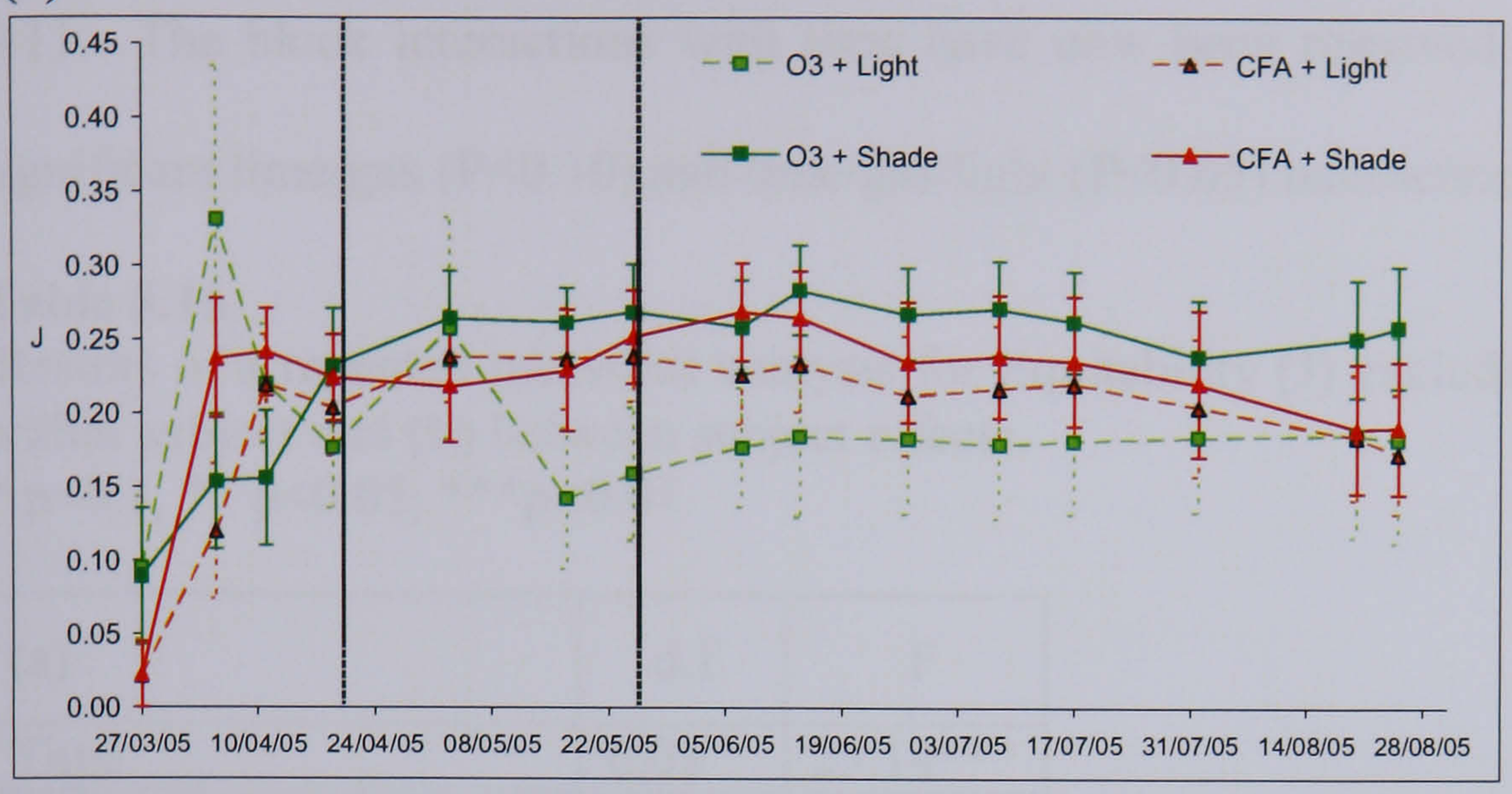
There was no significant overall effect of gas or light on either diversity or equitability. Both indices show a highly significant effect of time, there is an initial increase in H and J and then a steady decline (Fig 5.13 & 5.14). There were significant time/gas interactions for both indices ($p<0.01$). Fig 5.13 (a) shows the effect of gas

treatment over time for H'. The value in the control treatment becomes significantly greater than that in the ozone treatment early in the experiment, and this difference is maintained, although there is a tendency for the difference to decrease over time.

The time/gas/light interaction is also significant for changes in H' ($P<0.05$). Fig 5.13 (b) shows this clearly; a similar response over time is seen in the two control treatments and the shade ozone treatment. In contrast there is a marked decline in the value of H' following the first period of shade of the light ozone treatment (week 5), which therefore reaches a lower maximum than the other treatments. However over time these effects diminish.

J, shows a different response, and is complicated by various block interactions. These block interactions relate primarily to Block 2. Block 2 had two mesocosms with no species present and the other mesocosms were very erratic in the light ozone treatment with many fluctuations over time (5.14 (b)). The pattern of change in Block 2 seems to dictate the overall response shown in fig 5.14 (b) for the 'Light Ozone' treatment. This explains the significant interaction of gas/time ($P<0.05$) directly relating to the erratic changes of the ozone treatment which are not present in the control (Fig 5.14 (c)).

(a)



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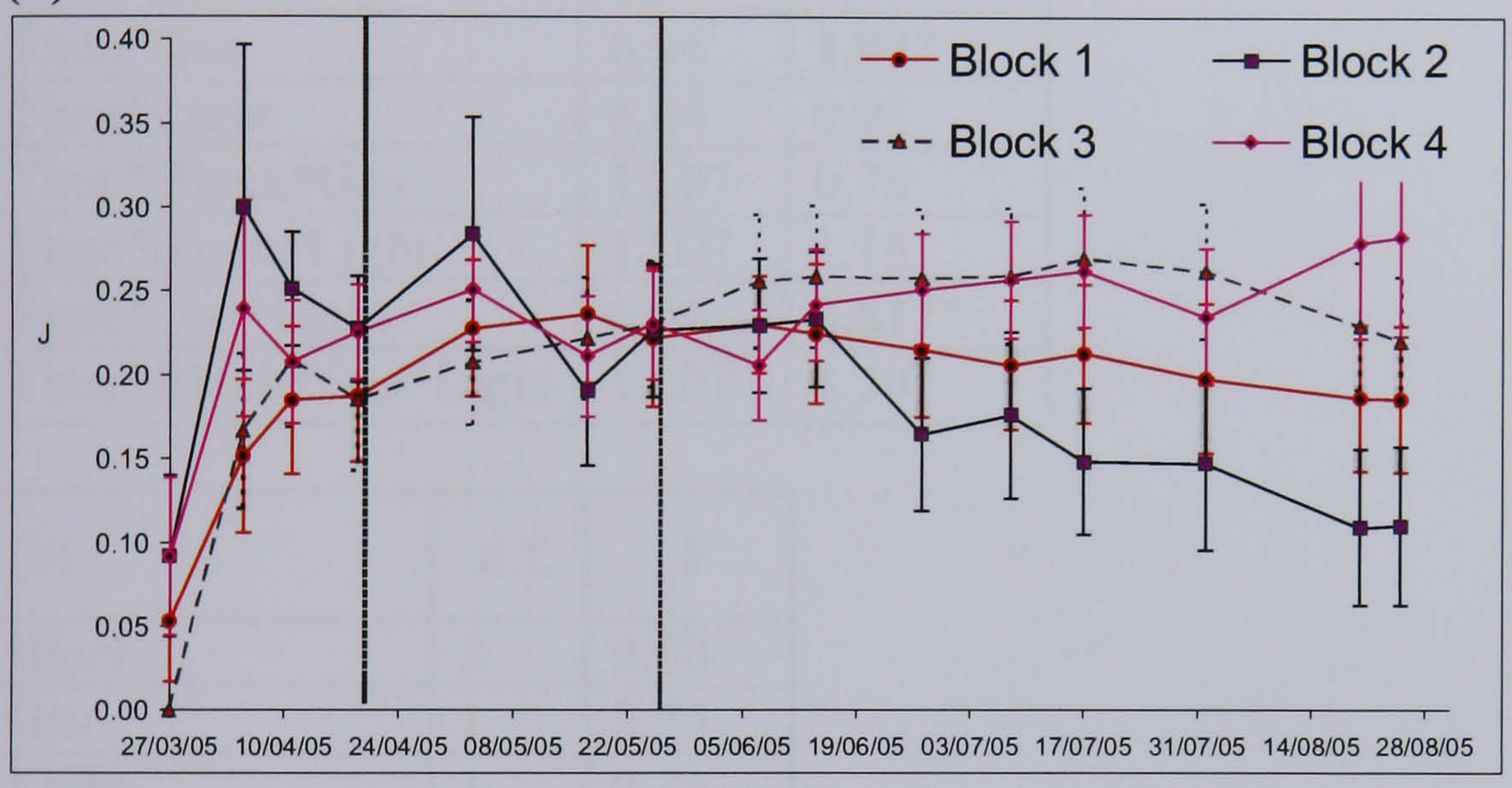

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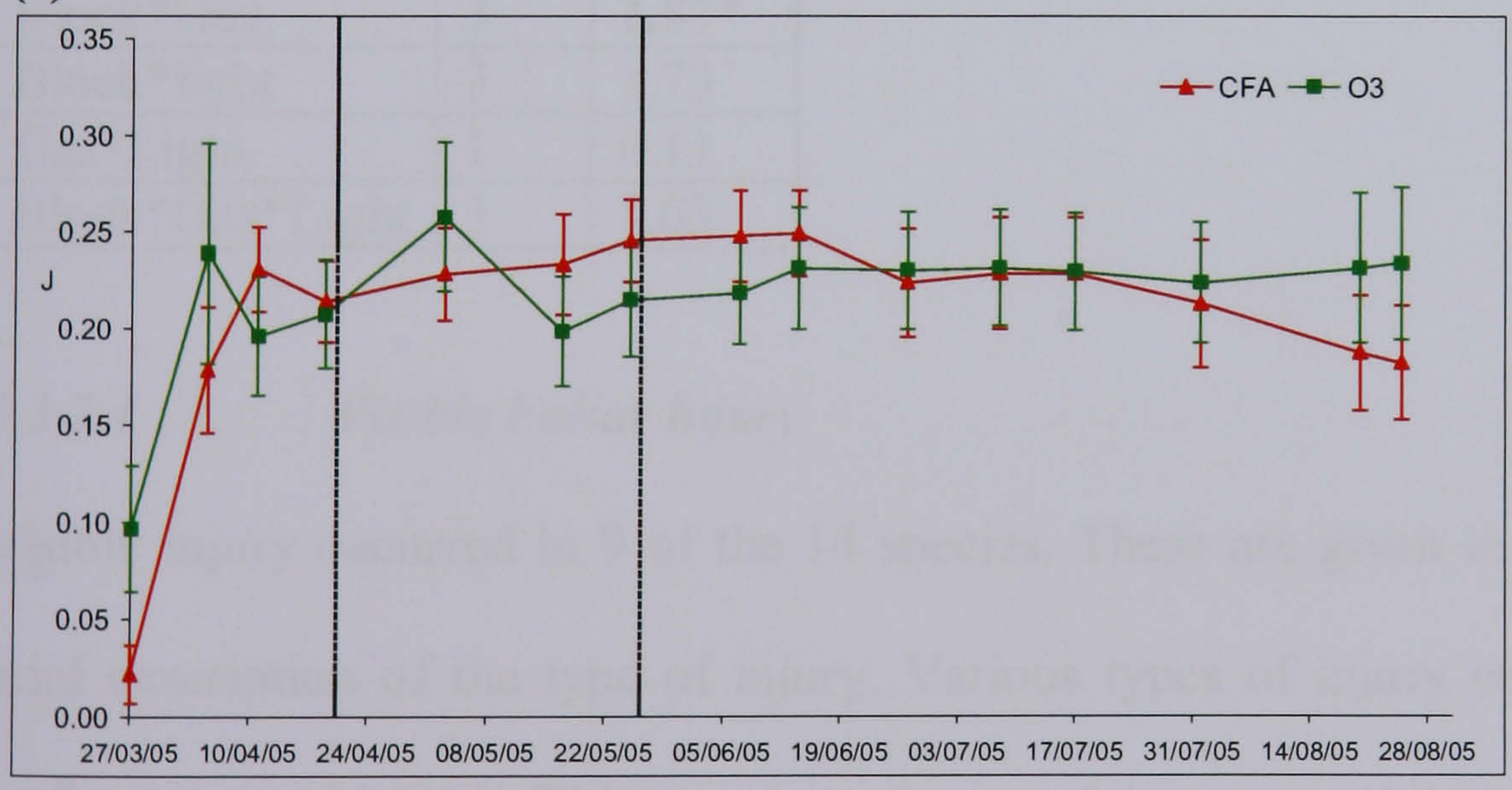


Fig 5.14

Mean Equitability (J): (a) for all four treatments; **(b)** averaged over treatments in each block; and **(c)** averaged over gas treatment, over time +/- 1s.e. First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light

The analysis for J was repeated removing Block 2; these results are given in Table 3.13. The block interactions with time have now been removed and there are still significant time/gas (P<0.10) and time/gas/light (P<0.05) interactions.

Table 5.16
Results of a repeated measures analysis for Equitability (J) excluding Block 2 **(a)** within subject and **(b)** between subject effects.
* p<0.1, ** p<0.05, ***p<0.01

(a)	d.f.	F
Time	6.03	23.14***
Time*Block	12.07	0.83
Time*Gas	6.04	**1.99***
Time*Light	6.04	0.91
Time*Block*Gas	12.07	0.79
Time*block*Light	12.07	1.18
Time*Gas*Light	6.04	**2.81****
Time*Block*Gas*Light	12.07	1.50

(b)	d.f.	F
Block	3	0.08
Gas	1	2.73
Light	1	0.25
Block*Gas	3	**2.87***
Block*light	3	1.73
Gas*Light	1	0.11
Block*Gas*Light	3	1.03

5.3.2.4 Visible Foliar Injury

Visible injury occurred in 9 of the 14 species. These are given in Table 5.17 with a brief description of the type of injury. Various types of injury were observed from senescence to chlorosis. This was dependent on the species exhibiting damage.

Table 5.18 gives the results of a repeated measures analysis for all types of foliar injury observed (Table 5.17), expressed as total percentage foliar injury. Fig 5.15 (a) shows the mean percentage foliar injury per treatment over time. There is a significant

effect of time (P<0.01). Visible injury first occurs in the ozone treatments in the first few weeks of the experiment before any shade has been applied; this is 05/04/05, the start of week 2 of ozone fumigation. There is also a significant time/gas interaction (P<0.01), as foliar injury increases over time in the ozone treatment.

Table 5.17
Species displaying visible injury during the fumigation period

Species	Stipple	Fleck	Chlorosis	Premature scensence	Reddenning
	Many small red, purple or other pigmented spots	Many small yellow Spots	Areas of leaf with a bright yellow colour	Leaf drop	areas or deep red or purple
Aegopodium podagraria			x		
Fragaria vesca				x	
Geum urbanum			x		
Glechoma hederacea		x			
Hypericum humifusum	x	x	x		x
Luzula campestris			x		
Mercurialis perennis			x		
Potentilla sterelis		x	x		
Rubus fructicosus (agg.)	x	x	x		
Viola riviana			x	x	

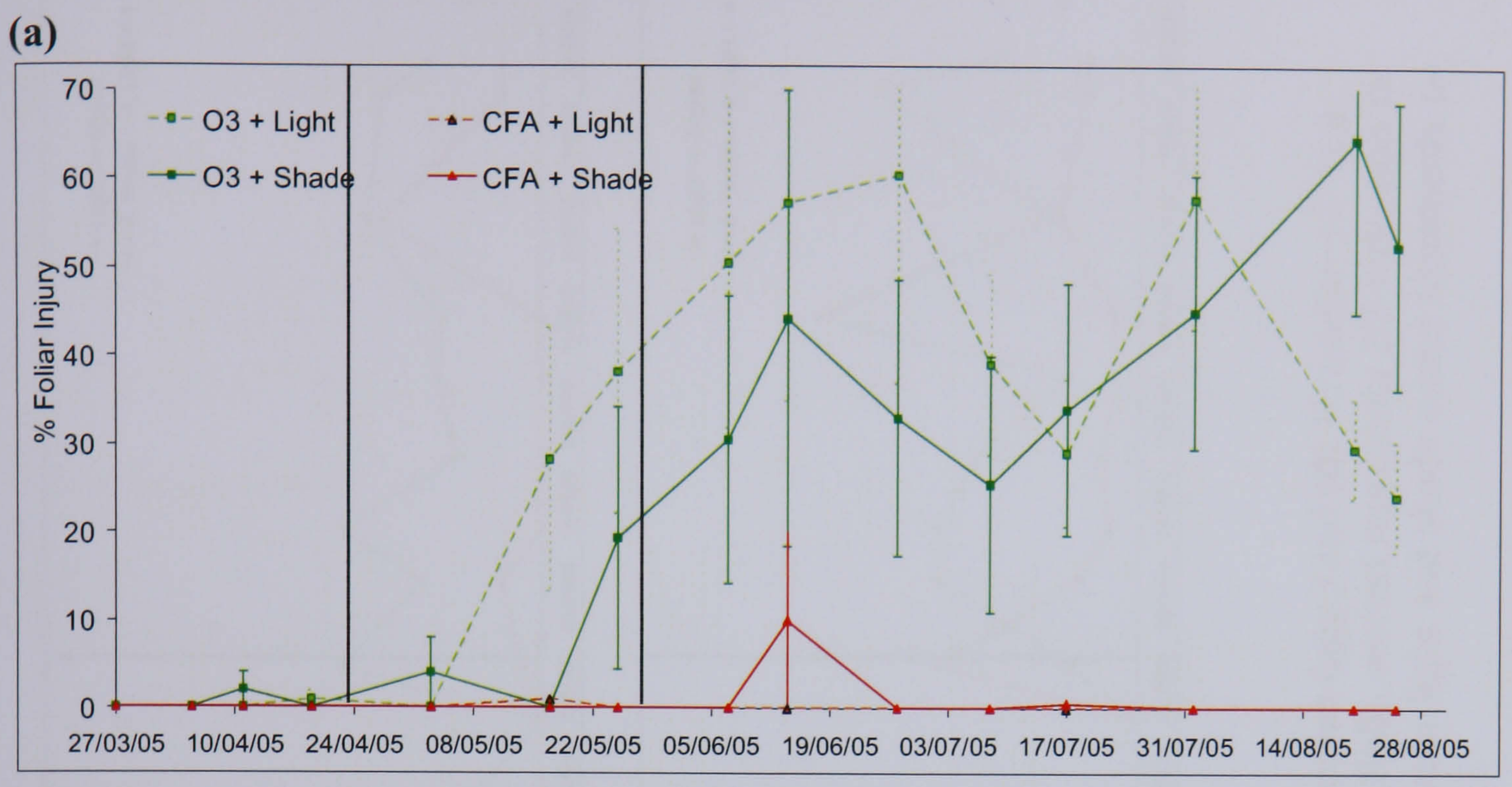
By the time the first shade was introduced, occurrence of injury and mean percentage visible injury had increased in the two ozone treatments. There is very little foliar damage in the control treatments, there is a small occurrence of injury in the shade control treatment following the second shade this was senescence on *V.riviana.* Fig 5.15 (a) shows clearly the significant overall effect of gas treatment (P<0.01) on the quantity of foliar injury.

Table 5.18
Results of a repeated measures analysis for percentage visible foliar injury **(a)** within subject and **(b)** between subject effects.
* = P<0.10, ** = P<0.05; *** = P<0.01

(a)	d.f.	F
Time	7.80	**6.94*** **
Time*Block	23.40	1.24
Time*Gas	7.80	**6.26*** **
Time*Light	7.80	1.49
Time*Block*Gas	23.40	1.09
Time*block*Light	23.40	0.99
Time*Gas*Light	7.80	1.47
Time*Block*Gas*Light	23.40	**1.51***

(b)	d.f.	F
Block	3	**3.12****
Gas	1	**31.29*** **
Light	1	0.00
Block*Gas	3	**2.97***
Block*light	3	1.04
Gas*Light	1	0.01
Block*Gas*Light	3	1.40

Fig 5.15 (b) is an illustration of the significant overall block effect (P<0.05). This graph clearly shows that each block responds differently, showing different levels of injury. Blocks 2 & 4 show the highest levels of damage. There is an overall effect of block/gas (P<0.10). Fig 5.16 shows the sum of individual species damage, over all four blocks, in the ozone treatments. Foliar injury to *H.humifusum* caused the large peaks in foliar injury in Block 2 and 4 (Fig 5.15 b & d).



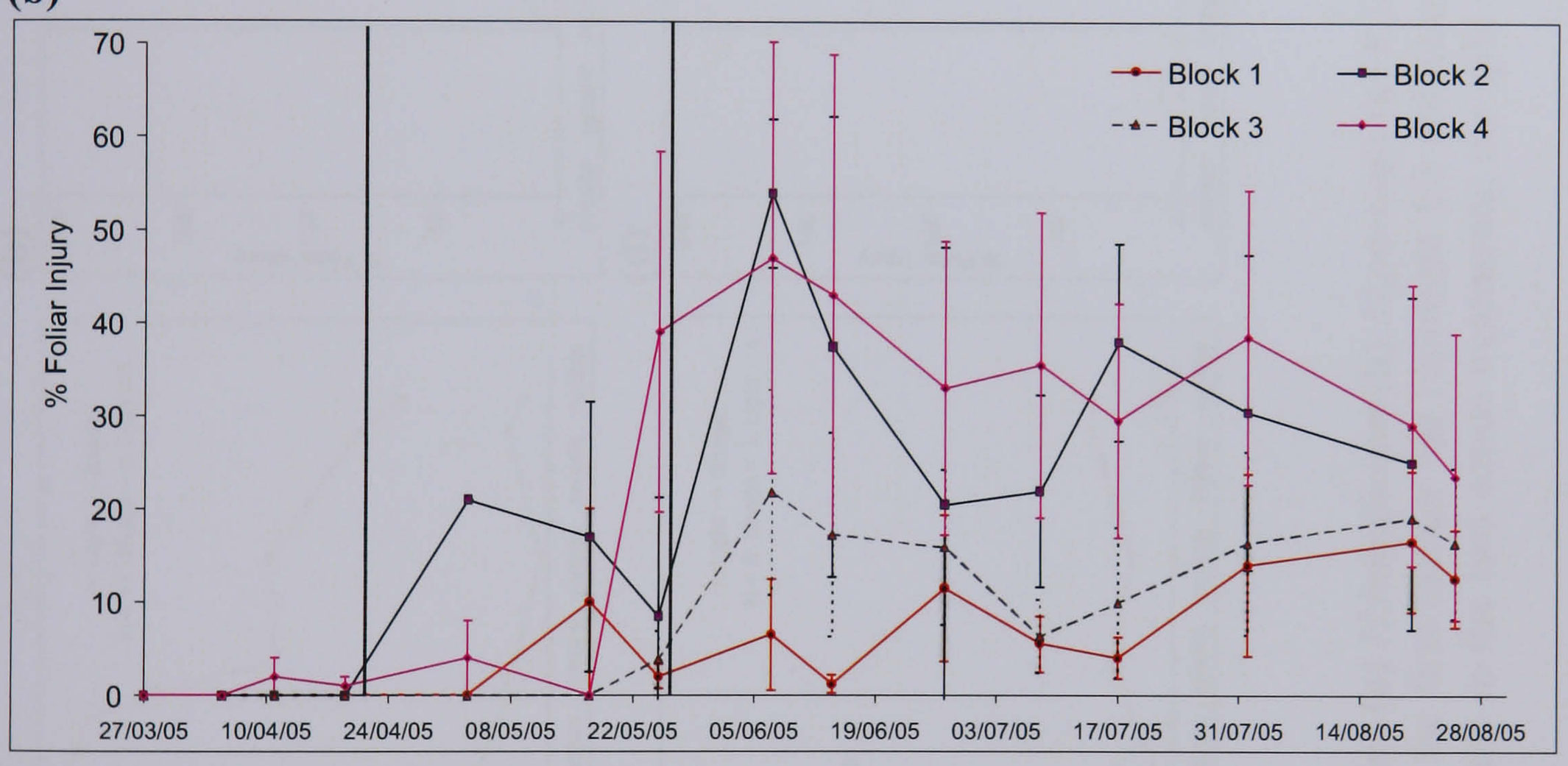


Fig 5.15
Mean percentage visible foliar injury (Table 5.17) **(a)** by treatment and **(b)** by block; Scale starts a day 1 of ozone fumigation; +/- 1s.e.
the first marker bar represent 1/3 reduction in light, second represents 2/3 reduction in light

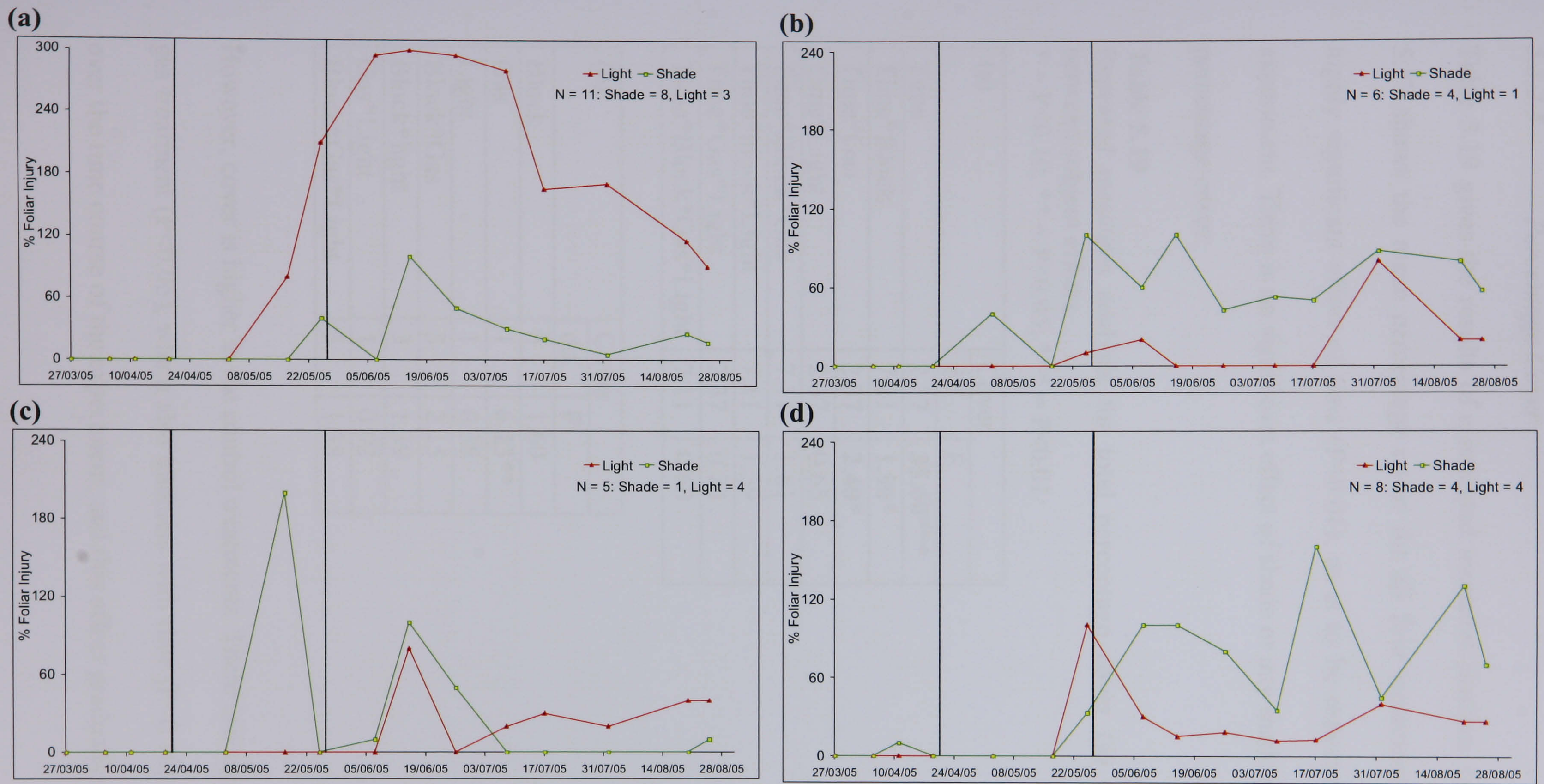


Fig 5.16
Mean foliar injury over time for four species **(a)** *H.humifusum* **(b)** *M.perrenis* **(c)** *R.fructicosus* (agg.) and **(d)** *V.riviana*: +/- 1s.e. Scale starts a day 1 of ozone fumigation, the first marker bar represent 1/3 reduction in light, second represents 2/3 reduction in light. **N** indicates the total number of plants in the two ozone treatments; the split between light and shade ozone treatments is also indicated.

Original in colour

5.3.2.5 Percentage Cover

Table 5.19 gives the results of a repeated measures analysis for percentage cover. Fig 5.17 shows the mean percentage cover for all four treatments over time. There is a highly significant effect of time ($P<0.01$), as is to be expected with growth over the experiment. There is no significant effect of shade or any shade/gas interactions on mean percentage cover.

Table 5.19
Repeated measures analysis for total percentage cover **(a)** within subject and **(b)** between subject effects
* = P<0.10, ** = P<0.05; *** = P<0.01

(a)	Cover	
	d.f..	F
Time	2.37	**35.60*** **
Time*Block	7.11	**1.96***
Time*Gas	2.37	**2.40***
Time*Light	2.37	0.65
Time*Block*Gas	7.11	1.61
Time*block*Light	7.11	1.59
Time*Gas*Light	2.37	0.24
Time*Block*Gas*Light	7.11	0.60

(b)	Cover	
	d.f.	F
Block	3	1.60
Gas	1	**6.23** **
Light	1	0.38
Block*Gas	3	2.15
Block*light	3	1.39
Gas*Light	1	0.02
Block*Gas*Light	3	1.38

However, cover is higher in the control treatments. There is a significant overall effect of gas treatment ($P<0.05$), which also interacts with time ($P<0.10$). Ozone decreases cover over the time course of the experiment, and this effect gradually increases over time. Fig

5.17 also suggests a difference between the light and shade treatments; shade treatments have slightly higher cover than the light treatments, but this is not significant.

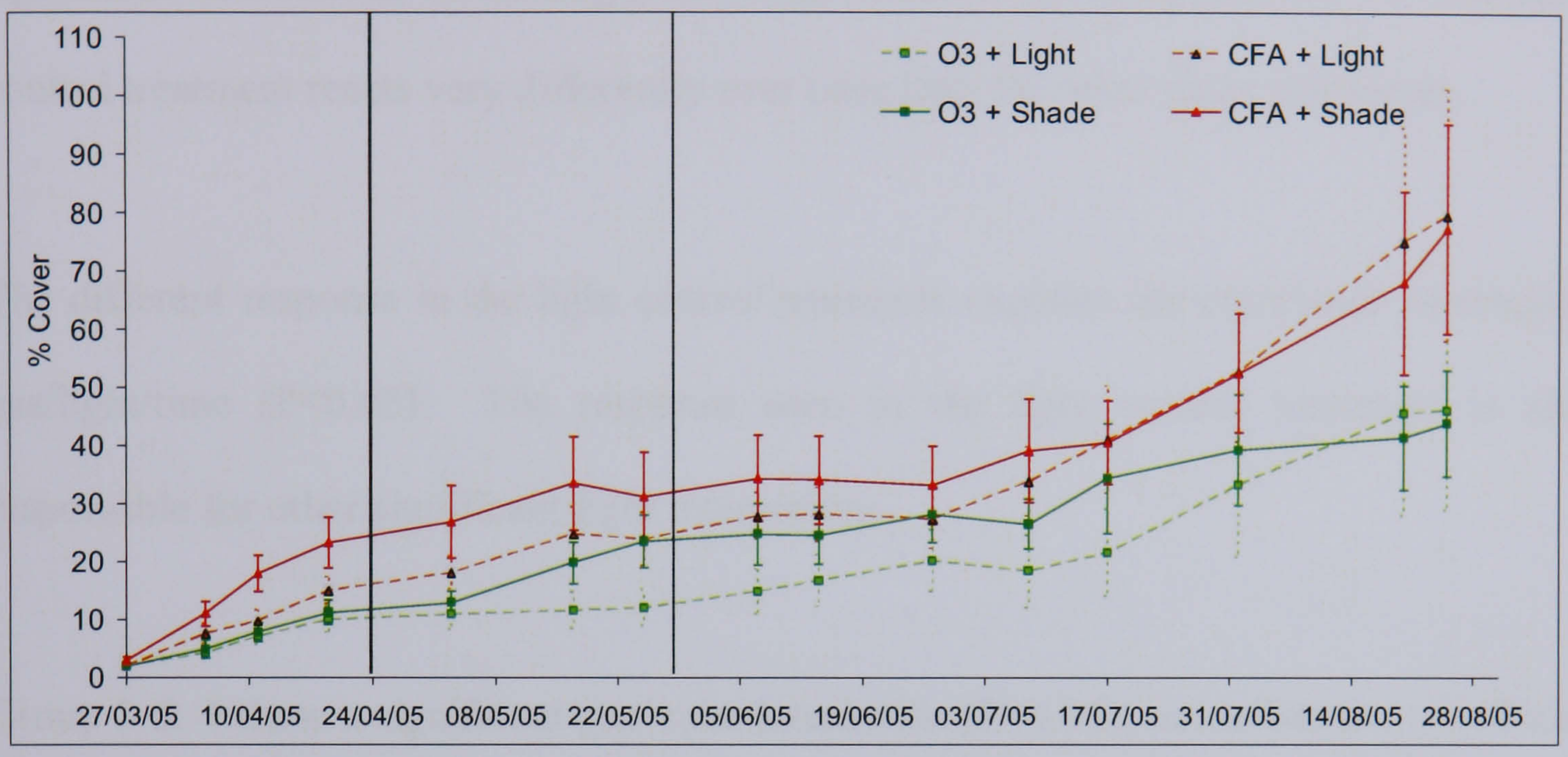


Fig 5.17
Mean percentage cover in each treatment over time +/- 1s.e.
First marker bar represent 1/3 reduction in light, second represents 2/3 reduction in light.

5.3.2.6 Percentage Cover by Ellenberg Index

Table 5.20 gives the results of a repeated measures analysis for percentage cover categorized by the Ellenberg index for light. Descriptions of the categories are given in Table 5.2. Fig 5.18 shows the mean percentage cover for all four treatments, again, ordered by the Ellenberg Index. Group 3 & 4 only had a few species present so they were combined. All categories show a significant effect of time ($P<0.01$).

Group 3 & 4

There is a significant overall effect of light treatment on this group ($P<0.05$) and significant interactions of time/light ($P<0.05$) and time/block/light ($P<0.05$). The light control treatment reacts very differently over time than the other three treatments.

The different response in the light control treatment explains the significant interaction gas/light/time ($P<0.05$). The response seen in the light control treatment is also responsible for other significant light interactions.

Group 3 & 4 show a significant gas/ time interaction ($P<0.05$); ozone has led to reduced cover in this group. There is also an overall gas effect irrespective of time ($P<0.10$). The two shade treatments show little growth until the introduction of the first shade, and then the shade appears to stunt any further growth. The light ozone treatment has similar cover to the two shade treatments. There is a significant light/time interaction ($P<0.05$), that seems to relate to these changes, with increasing difference between the light control treatment and the other three treatments.

Table 5.20
Repeated measures analysis for percentage cover by Ellenberg Index **(a)** within subject and **(b)** between subject effects
* = P<0.10, ** = P<0.05; *** = P<0.01

(a)	3 & 4		5		6		7	
	d.f.	F	d.f.	F	d.f.	F	d.f.	F
Time	2.47	**9.52*** **	2.06	**4.55*** **	2.27	**8.74*** **	2.07	**13.77*** **
Time*Block	7.42	1.64	6.17	0.99	6.82	0.31	6.22	1.04
Time*Gas	2.47	**2.94****	2.06	0.71	2.27	0.66	2.07	0.56
Time*Light	2.47	**4.33****	2.06	0.30	2.27	1.43	2.07	0.23
Time*Block*Gas	7.42	**2.07***	6.17	0.77	6.82	0.84	6.22	0.61
Time*block*Light	7.42	0.58	6.17	0.95	6.82	0.40	6.22	0.43
Time*Gas*Light	2.74	**3.00****	2.06	0.09	2.27	0.39	2.07	0.42
Time*Block*Gas*Light	7.42	1.22	6.17	0.98	6.82	0.68	6.22	0.72

(b)	d.f.	F			
		3&4	5	6	7
Block	3	2.13	1.36	0.22	0.56
Gas	1	**3.42***	1.00	**3.92***	0.00
Light	1	**4.73****	0.36	**4.35****	0.54
Block*Gas	3	**3.95****	0.90	0.95	0.70
Block*light	3	0.12	1.12	0.31	0.45
Gas*Light	1	0.75	0.12	0.28	0.81
Block*Gas*Light	3	1.46	0.15	0.85	0.94

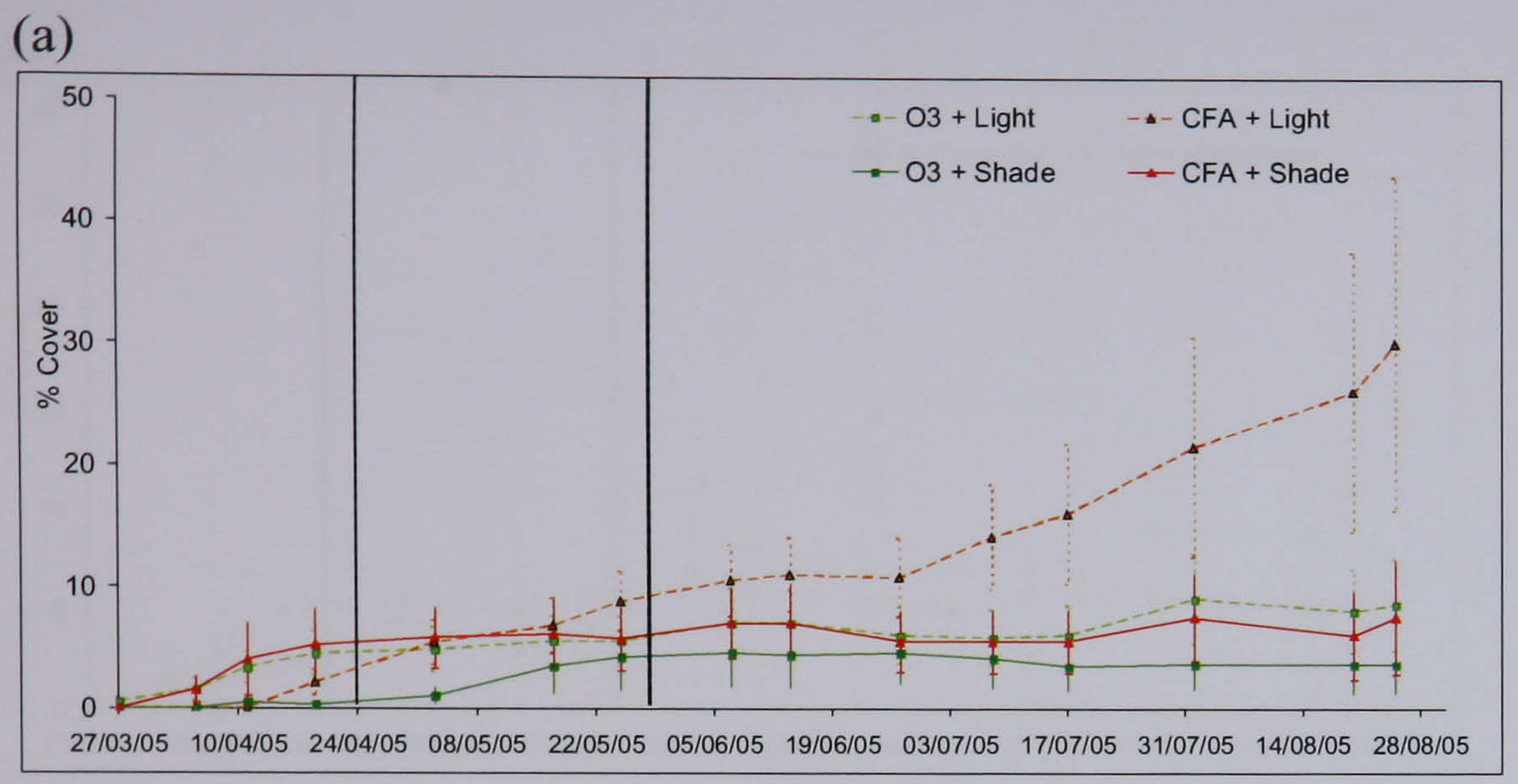
There is a significant block/gas interaction ($P<0.05$) and a time/block/gas ($P<0.10$) interaction. Fig 5.19 (a-d) maps the response of the four blocks over time, averaged showing the responses of the two gas treatments for the species present in each Block. Blocks 1 and 4 show a higher mean for the ozone treatment and Blocks 2 & 3 show higher means for the controls. It is clear that response depends on the species present; *M.perrenis* performed well in all blocks and dominated the group. The ozone treatment in Block 2 only had one individual present from this group of species, and hence had a very low cover. Except for block 1 *M.perrenis* had greater cover in the control treatments within the block. In contrast, *G.urbanum* was much less frequent and caused large differences in block to block responses. Furthermore, although it was present in the ozone treatment mesocosms, it was often short lived.

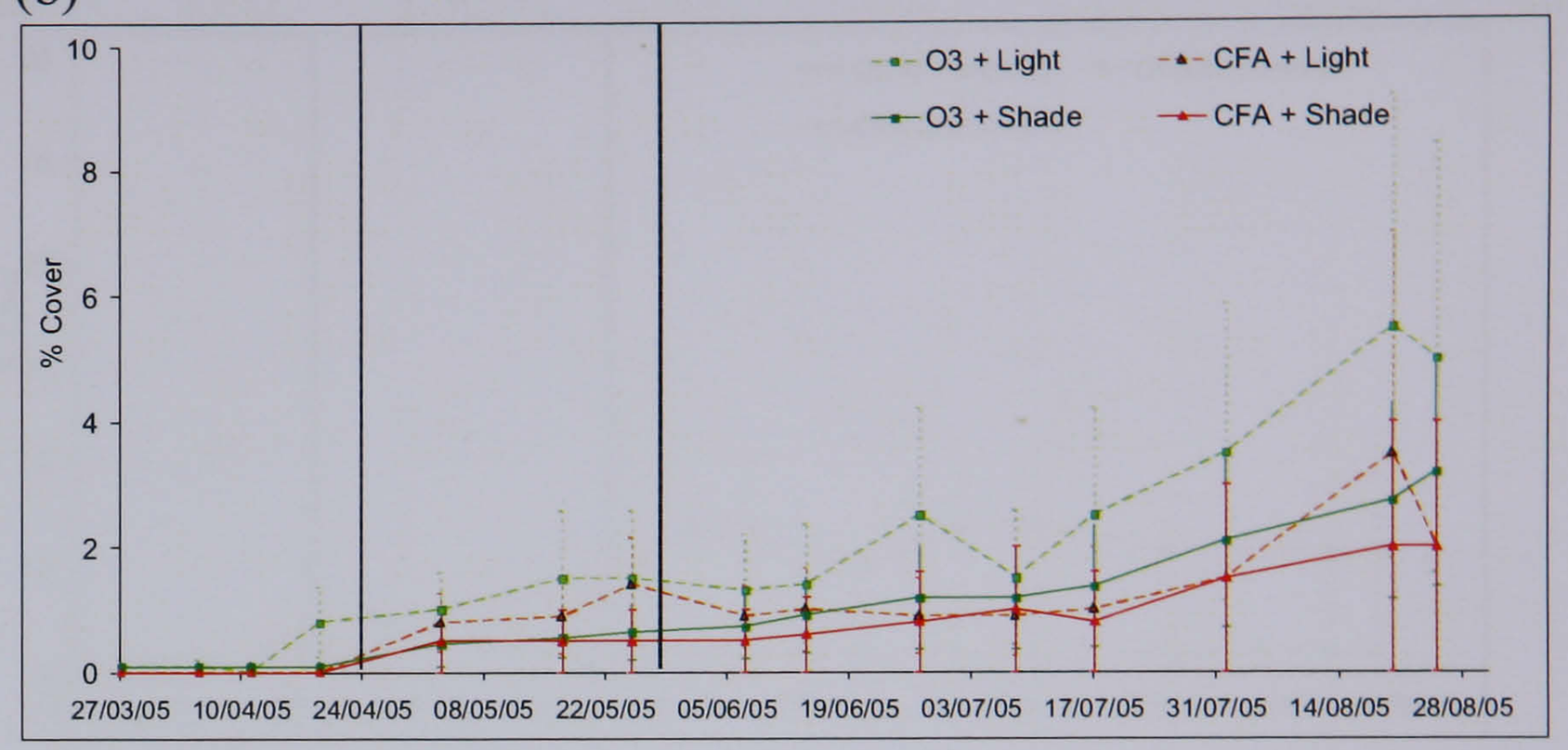
Groups 5 and 7

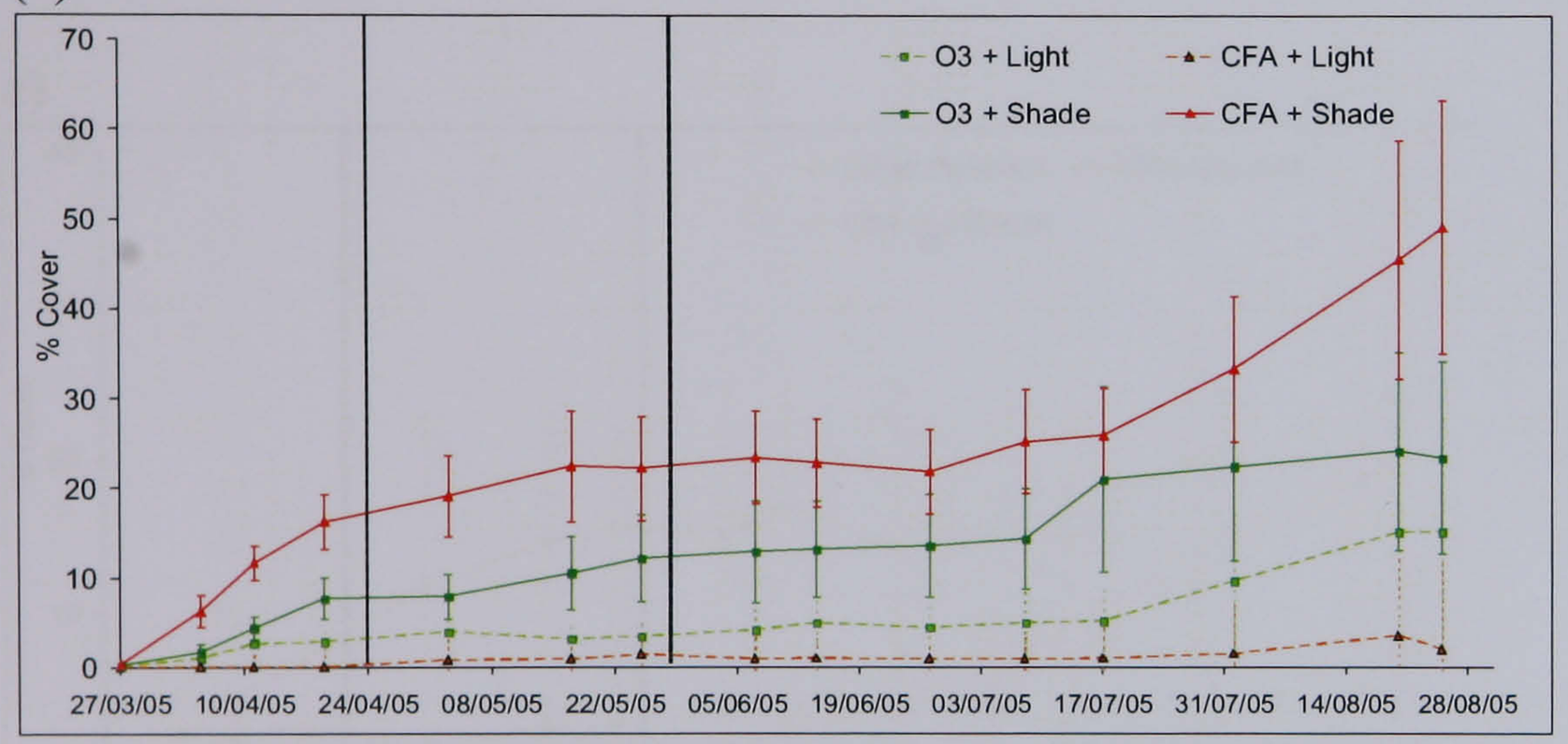
There were no treatment significant effects on cover for groups 5 and 7. The species in this group are too small in number, and cover values are low and are particularly variable.

Group 6

There are effects of ozone ($P<0.10$) and light ($P<0.05$) with no interactions for this group. Ozone caused reduced cover in this group, as in groups 3 & 4. The shade treatments caused an increase in cover, in contrast to groups 3 & 4.







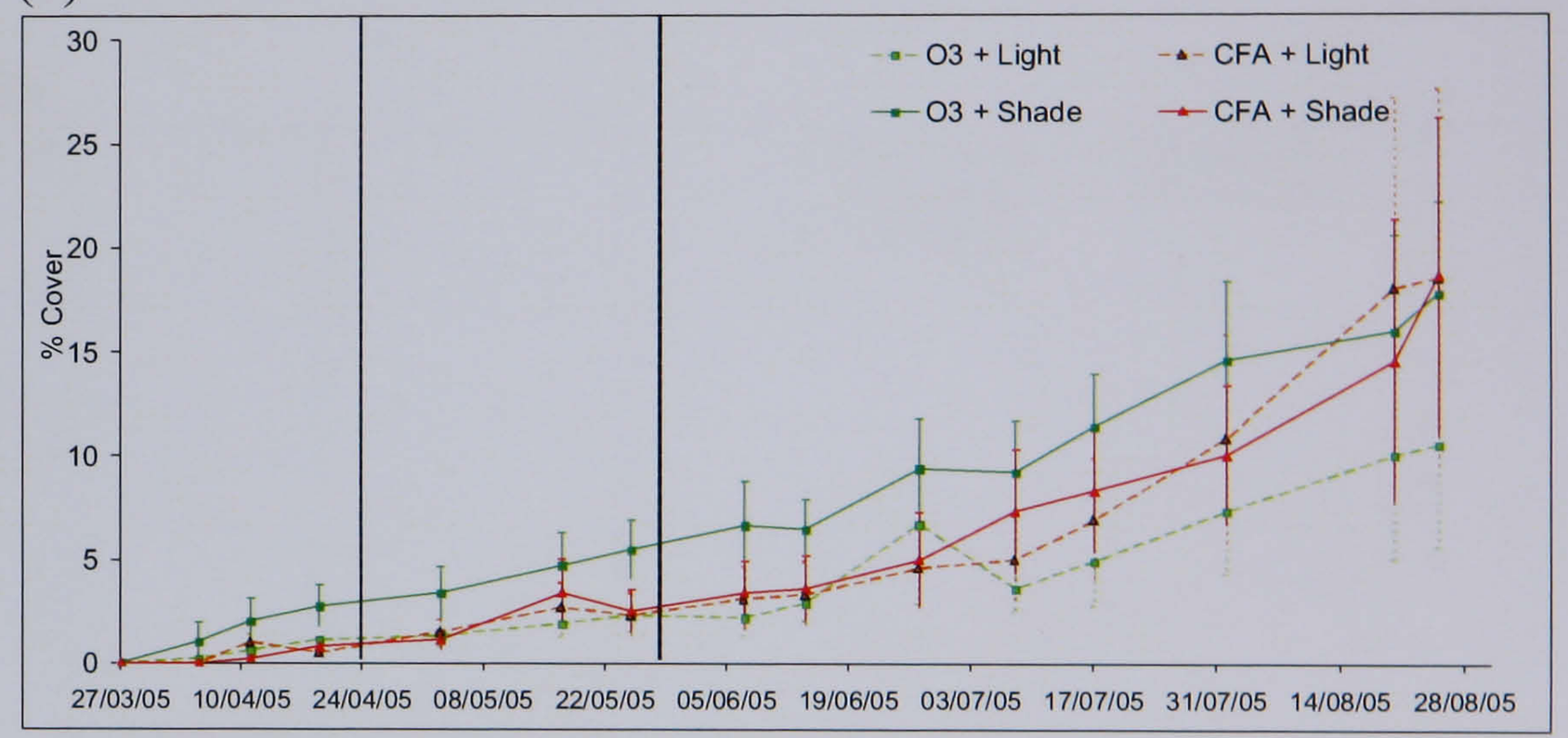
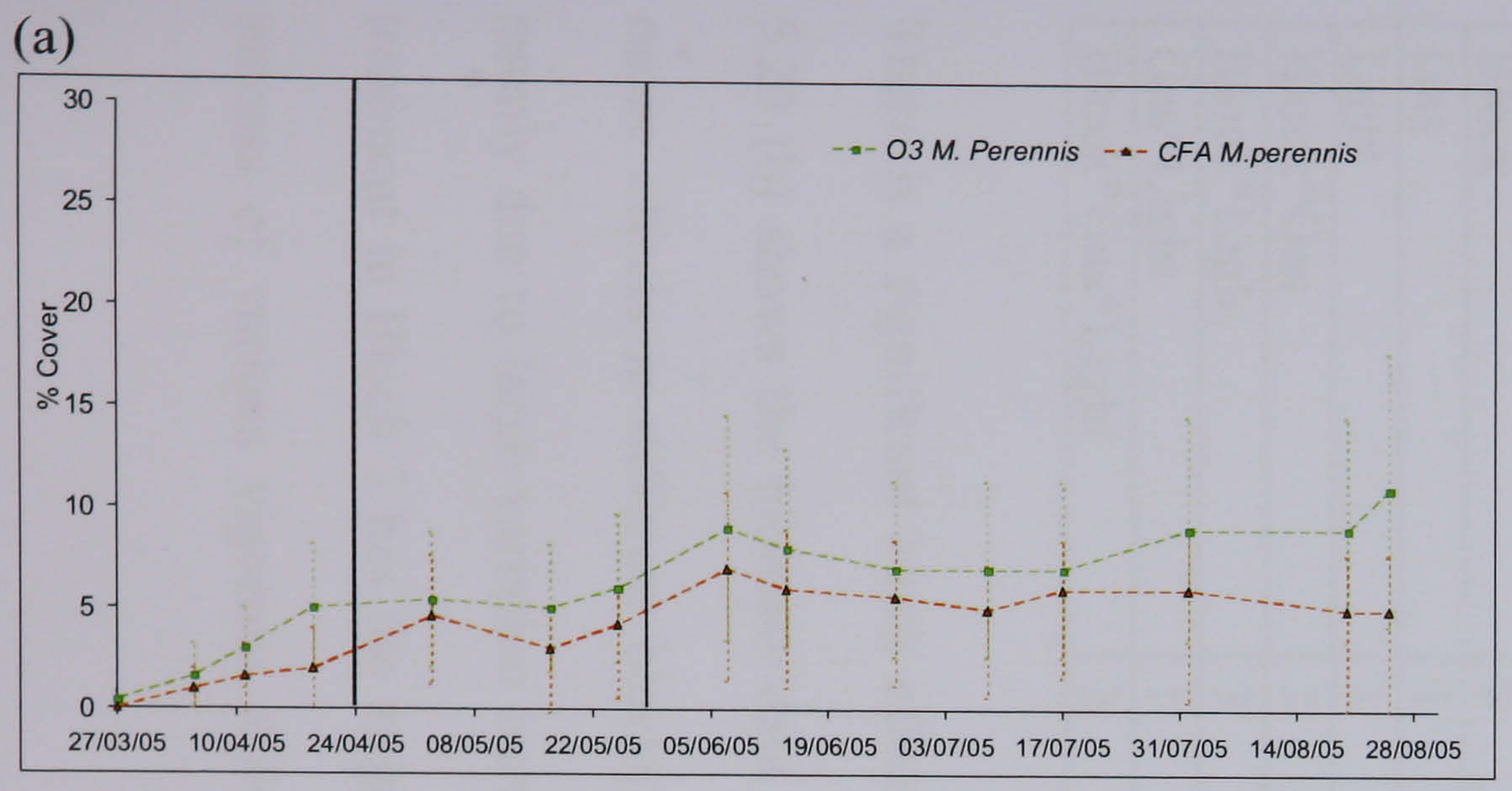
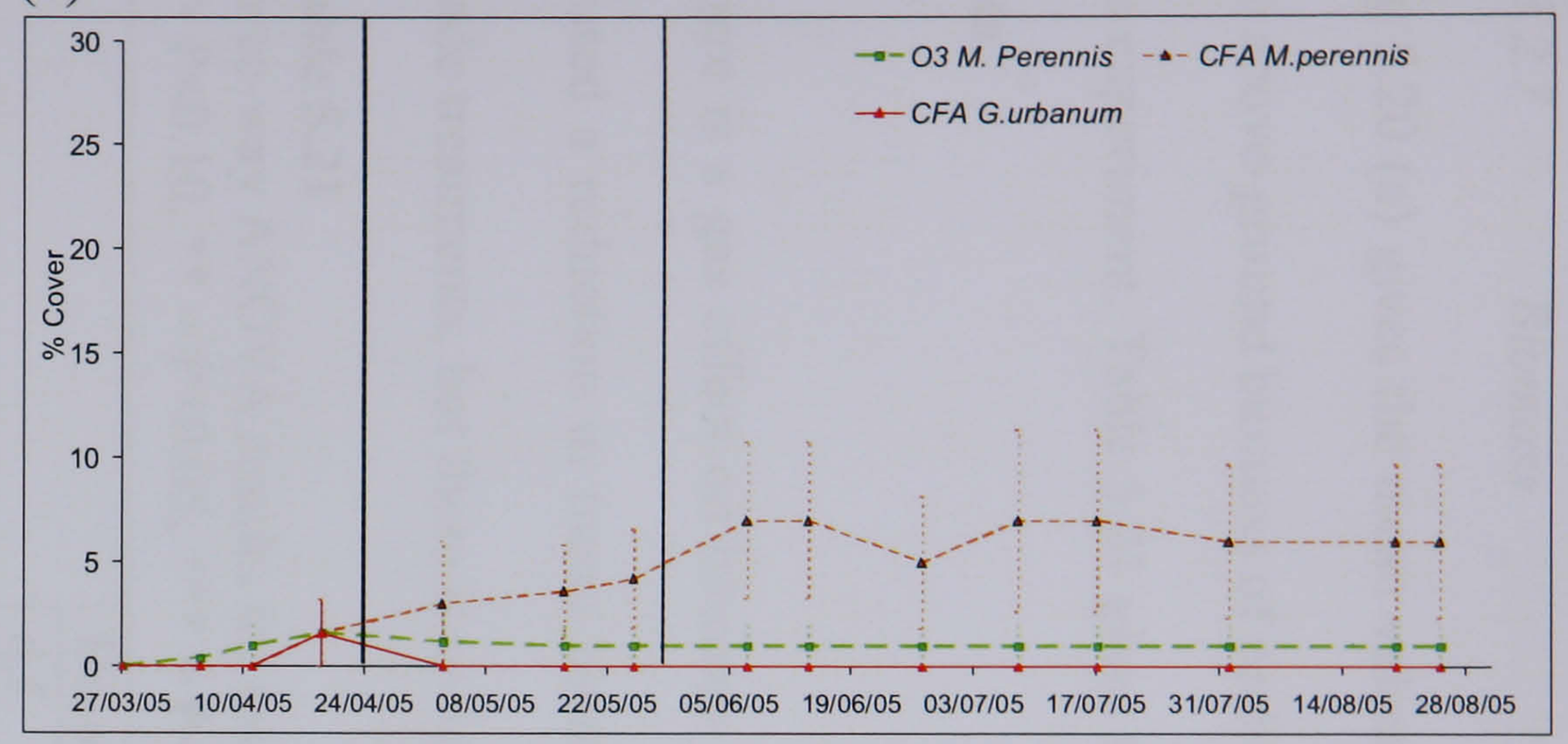
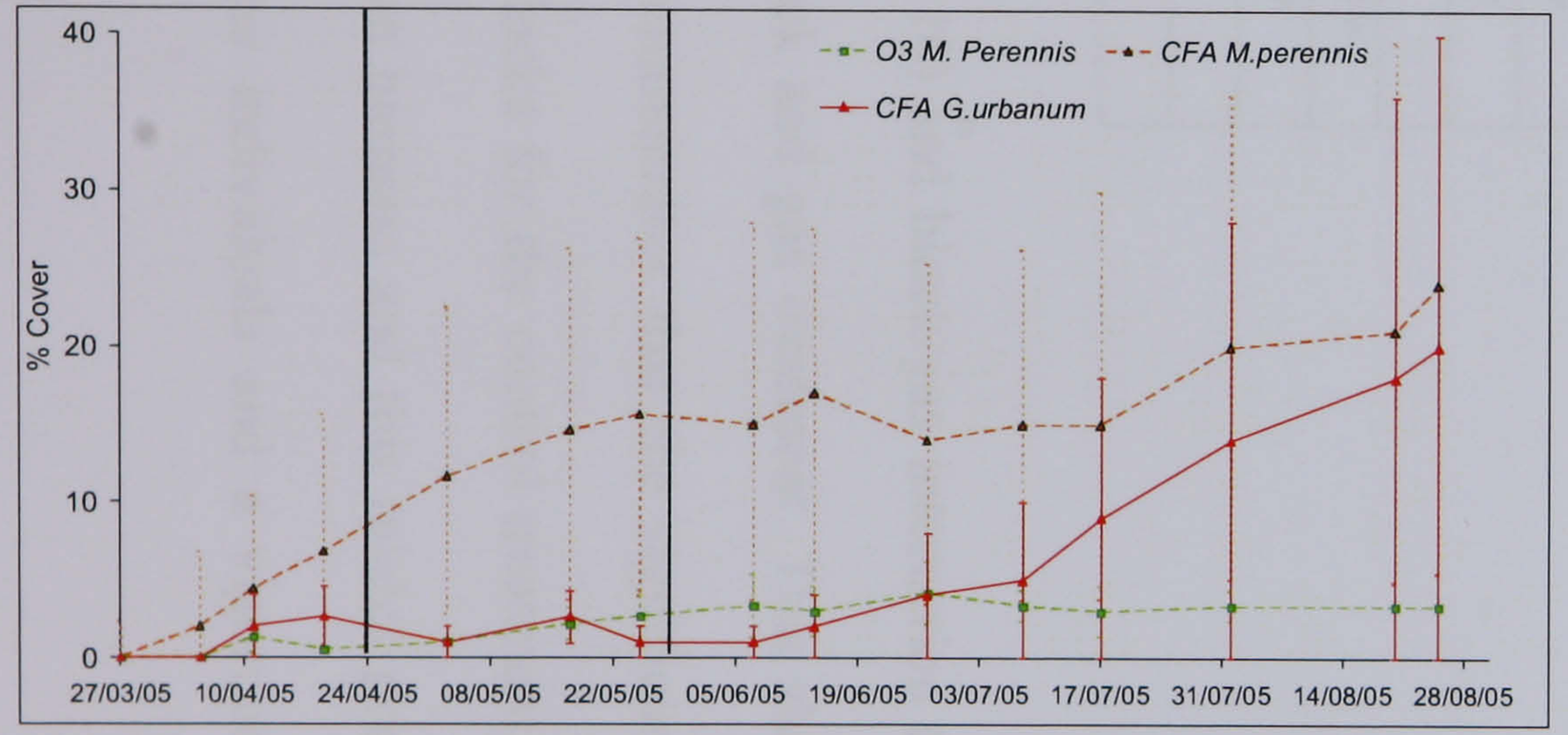


Fig 5.18
Mean percentage cover over time for the four treatments in different Ellenberg categories; **(a)** 3 & 4 (most shade tolerant), **(b)** 5, **(c)** 6, **(d)** 7 (sun species), First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light.







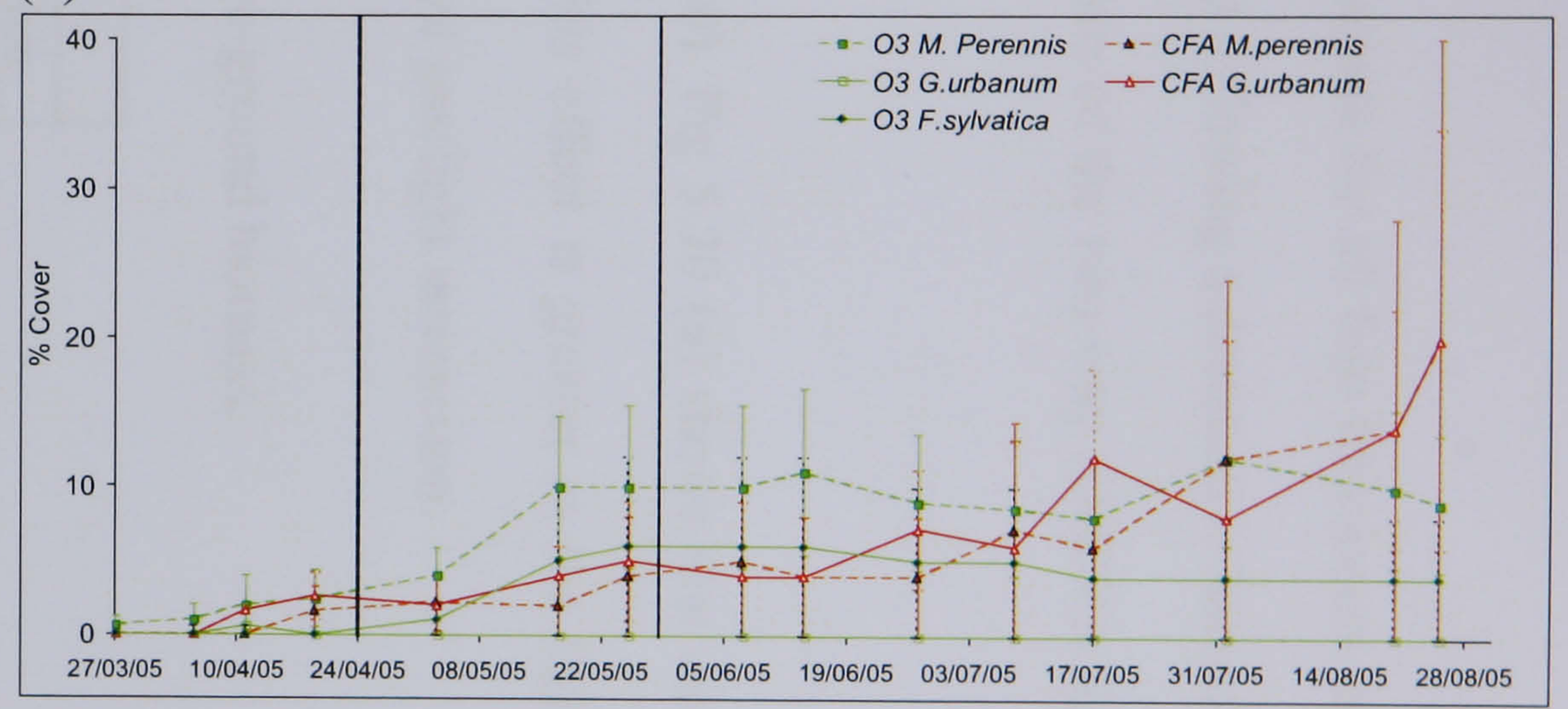


Fig 5.19
Mean percentage cover over time for Ellenberg Categories 3 and 4 averaged by gas treatment and species: **(a)** Block 1, **(b)** Block 2, **(c)** Block 3, **(d)** Block 4; First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light; Error bars represent +/- 1.s.e.

5.3.2.7 Biomass

Fig 5.20 (a) gives the mean values of biomass for all four treatments calculated from the above-ground biomass of each species following a destructive harvest at the end of the experiment. Table 5.21 gives the results of the two-way ANOVA for the biomass data.

There is a gas effect on biomass (P<0.10). Fig 5.20 (a) shows that ozone treatment caused a reduction in mean biomass. This effect is greater in the light than in the shade treatments, but there is no significant gas/light interaction.

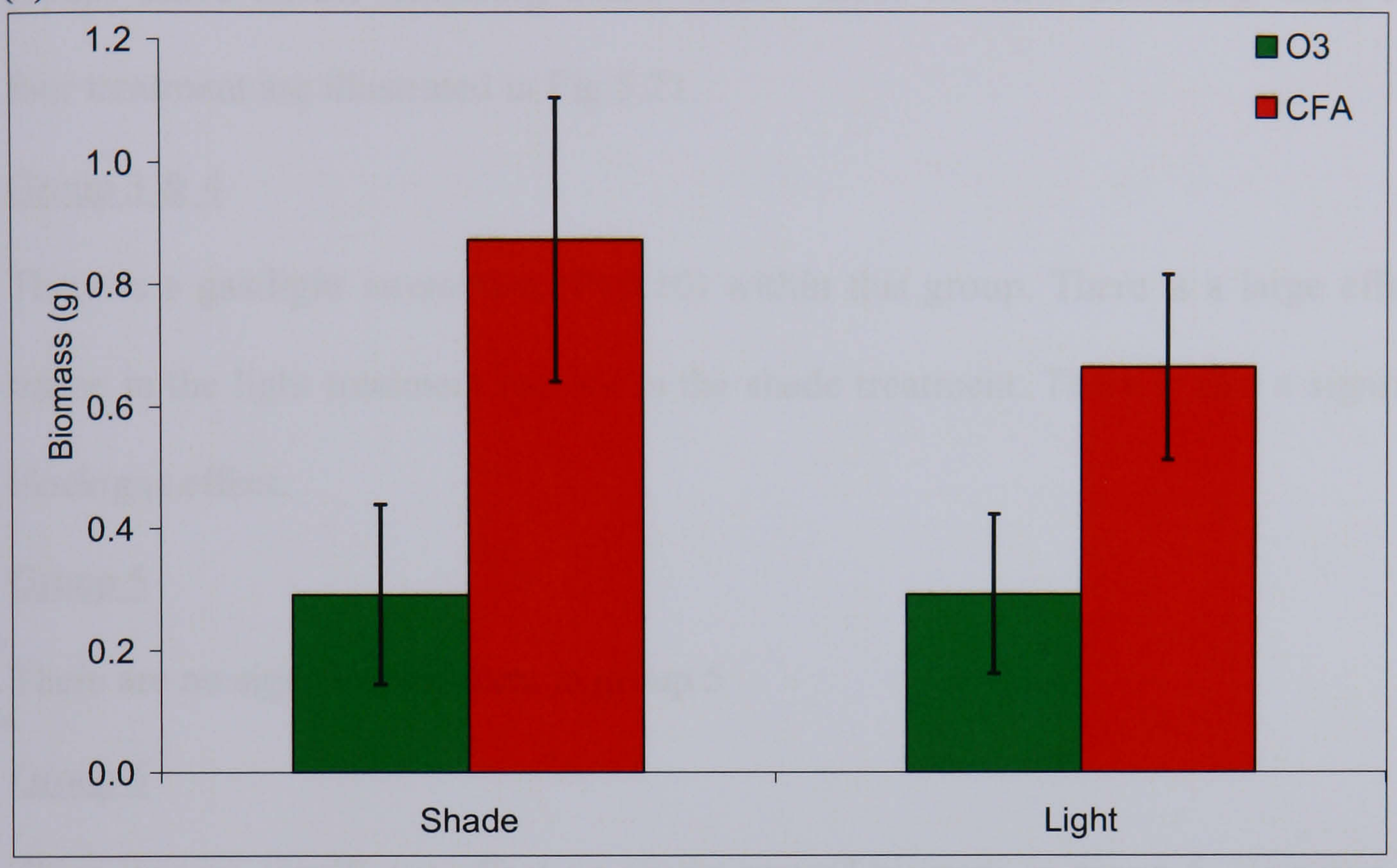
Table 5.21
Three-way ANOVA results for total above-ground biomass.
* = P<0.10, ** = P<0.05; *** = P<0.01

	Biomass	
	d.f.	F-Value
Block	3	**2.43***
Gas	1	**3.44***
Light	1	1.93
Block*Gas	3	**3.75****
Block*Light	3	**1.78***
Gas*Light	1	1.97
Block*Gas*Light	3	0.42

There is a significant block effect (P<0.10) and block/gas interaction (P<0.05). Fig 5.20 (b) shows the biomass data by block and gas treatment. This shows different ozone effects in different blocks and demonstrates that the significant effect was mainly due to large variation between blocks for the control treatment. The control treatment in Block 3 has the largest mean biomass and this relates primarily to the success of various vigorous *R.fructicosus* individuals and a vigorous *G.urbanum*

within this treatment. The ozone treatment in this block also had vigorous *R.fructicosus* but the biomass values were lower.

(a)



(b)

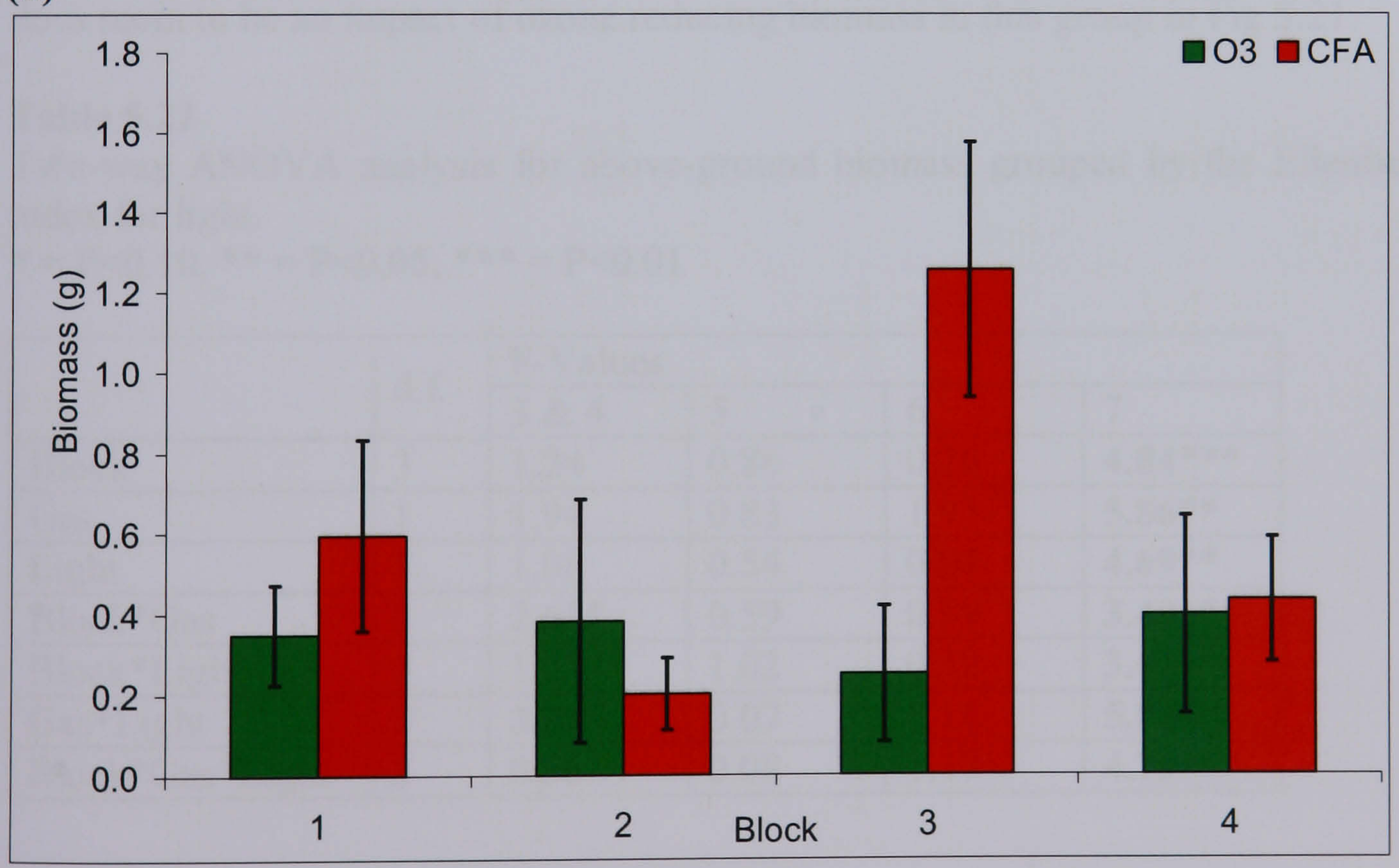


Fig 5.20
Mean **(a)** total live above-ground biomass (g) by treatment and **(b)** total live above-ground biomass by block and gas treatment

Original in colour

5.3.2.8 Biomass by Ellenberg Index

Table 5.22 gives the results of two-way ANOVA tests for above-ground biomass in groups based on the Ellenberg index. Mean values for each Ellenberg value for all four treatment are illustrated in Fig 5.21.

Group 3 & 4

There is a gas/light interaction (P<0.10) within this group. There is a large effect of ozone in the light treatment but not in the shade treatment. There is also a significant block/gas effect.

Group 5

There are no significant effects in group 5

Group 6

There was no significant effect on above-ground biomass in group 6. However there does seem to be an impact of ozone reducing biomass in this group in Fig 5.21.

Table 5.22
Two-way ANOVA analysis for above-ground biomass grouped by the Ellenberg index for light.
* = P<0.10, ** = P<0.05; *** = P<0.01

	d.f.	F-Values			
		3 & 4	5	6	7
Block	3	1.24	0.86	0.76	**4.84*****
Gas	1	1.94	0.83	1.93	**5.86****
Light	1	1.08	0.54	0.04	**4.69****
Block*Gas	3	**2.63***	0.59	0.89	**3.40****
Block*Light	3	1.03	1.02	0.32	**3.62****
Gas*Light	1	**3.33***	0.07	1.18	**5.00****
Block*Gas*Light	3	0.56	0.08	1.17	**4.75*****

Group 7

All biomass values are similar for group 7, except those of the light control treatment. As for group 3 & 4, this leads to large reduction in biomass by ozone in the light, but not in the shade, treatment. Gas, light and gas/light interactions are all significant for this group, showing clearly how this one treatment differs from the others.

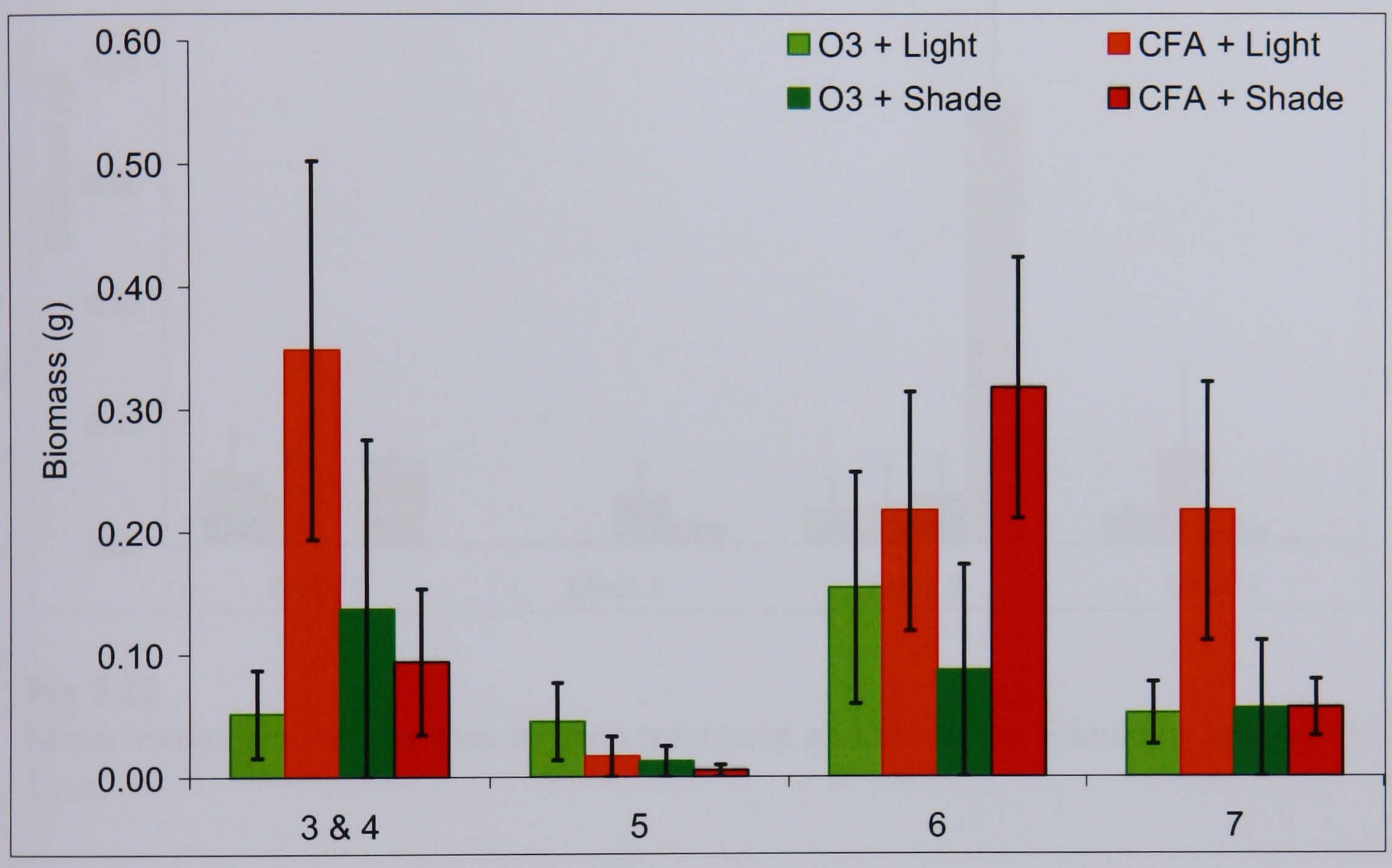


Fig 5.21
Mean total above-ground biomass per treatment in different groups based on the Ellenberg index for light +/- 1.s.e.

In terms of block interactions, Block 3 is significantly different from Block 2 ($P<0.05$) and Block 4 ($P<0.10$). Fig 5.22 shows the mean biomass for group 7 by block, showing that Block 3 is very different, especially the shade control group which has a very large mean for this block. There are many differences between treatments and blocks which make it difficult to distinguish any major effects. However it is clear that

ozone treatments consistently have low biomass values, whereas the control treatments have higher values.

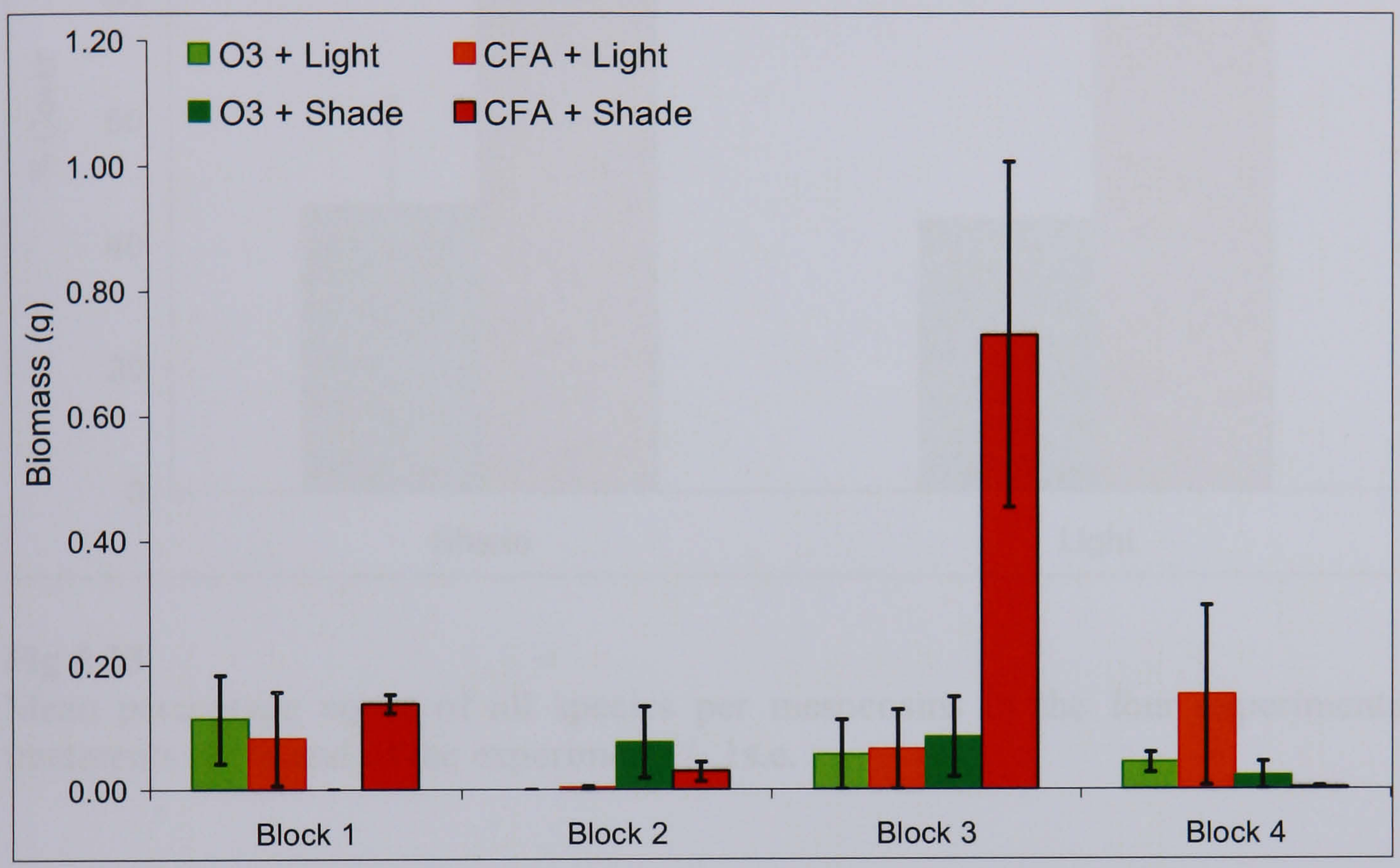


Fig 5.22
Mean above-ground biomass in each treatment and block for Ellenberg group 7 +/- 1.s.e.

5.3.2.9 Final Percentage Cover

Fig 5.23 shows the mean percentage cover of all species, in the four treatments at the end of the experiment, and Table 5.23 gives the results of an ANOVA of the data. There is a significant effect of gas treatment on the final percentage cover values ($P<0.05$). Fig 5.23 clearly demonstrates that the control treatments have a significantly higher mean total cover than the ozone treatments. There are no significant interactions with block or light, but there is a small overall effect of block ($P<0.10$)

Original in colour

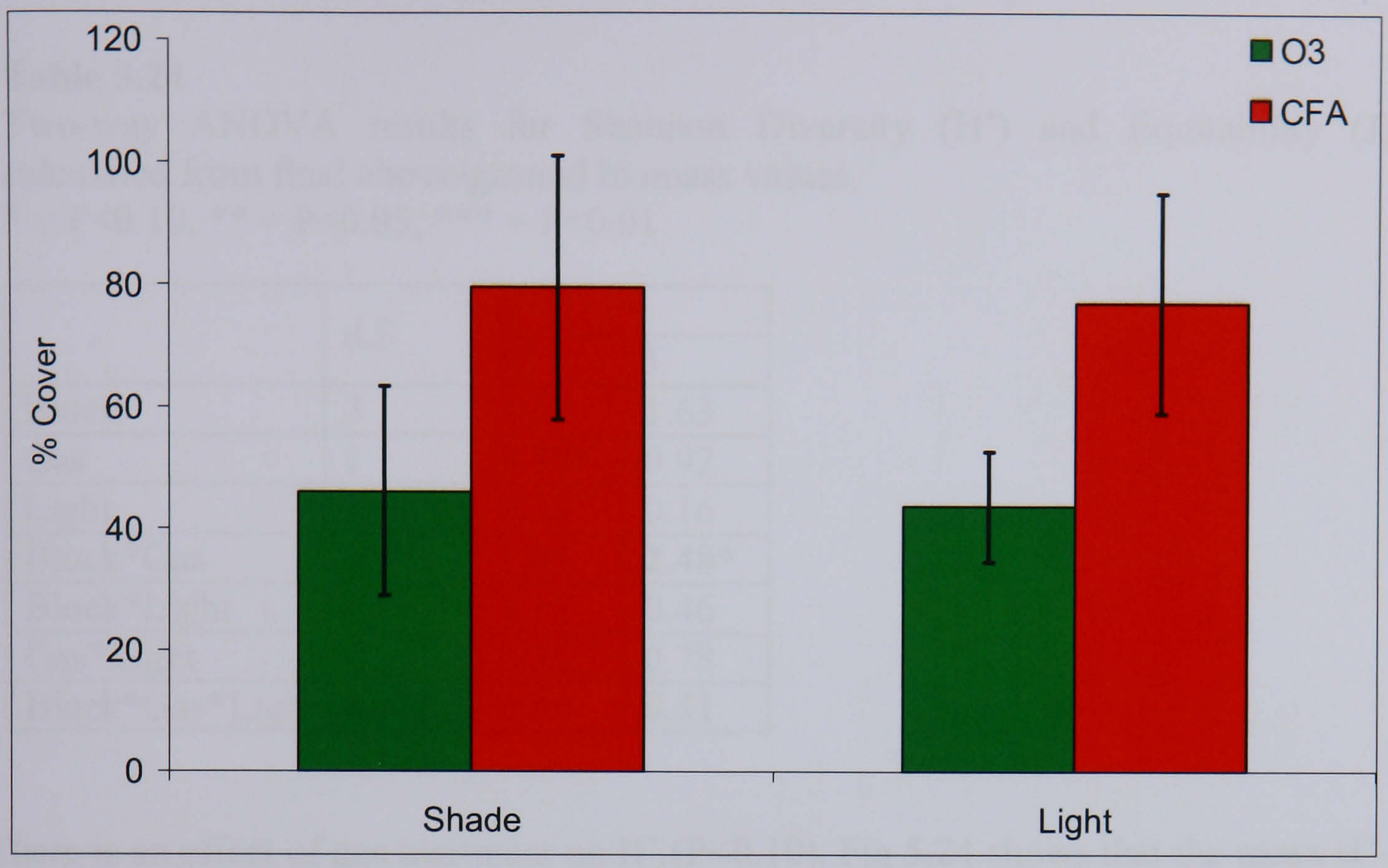


Fig 5.23
Mean percentage cover of all species per mesocosms in the four experimental treatments at the end of the experiment+/- 1s.e.

5.3.2.10 Shannon diversity Index and Equitability – Biomass

Table 5.24 gives the two-way ANOVA for H' and J calculated from biomass data. Fig 5.24 gives the mean values per treatments of (a) H' and (b) J. The effect of light was not significant for either parameter.

Table 5.24
Two-way ANOVA results for Shannon Diversity (H') and Equitability (J) calculated from final above-ground biomass values.
* = P<0.10, ** = P<0.05; *** = P<0.01

	d.f.	F	
		H'	J
Block	3	1.17	1.63
Gas	1	**2.95***	0.92
Light	1	0.38	0.16
Block*Gas	3	1.99	**2.48***
Block*Light	3	1.11	0.46
Gas*Light	1	0.51	0.78
Block*Gas*Ligh	3	0.84	0.31

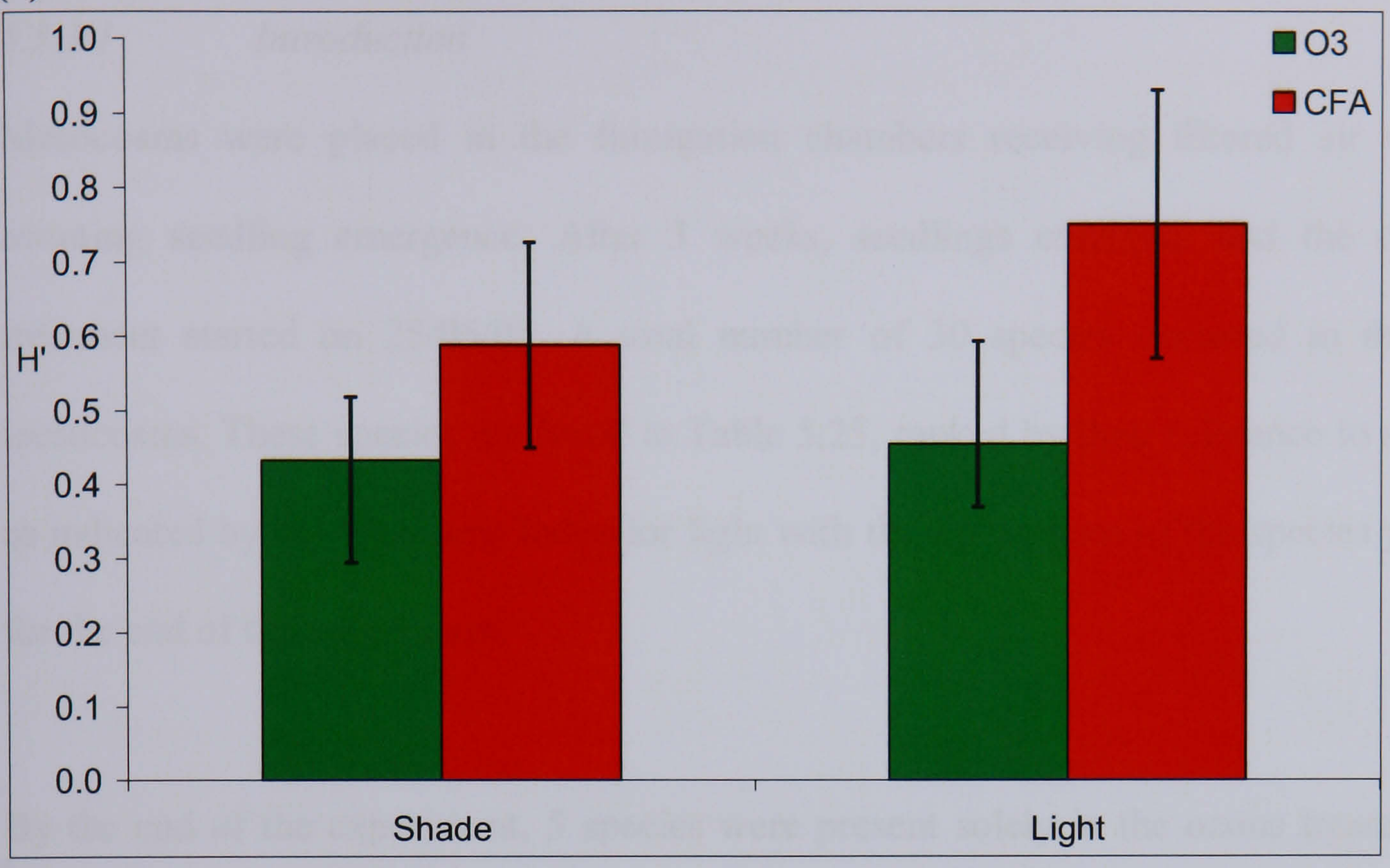
There is an effect of gas treatment on H' (P<0.10). Fig 5.24 shows that the mean H' is lower in the ozone treatments. There is a small block/gas interaction for J (P<0.10), but no overall effect of gas treatment.

Table 5.23
Two-way ANOVA results for final total cover.
* = P<0.10, ** = P<0.05; *** = P<0.01

	d.f	% Cover
Block	3	**2.32***
Gas	1	**4.68****
Light	1	0.74
Block*Gas	3	2.03
Block*Light	3	1.49
Gas*Light	1	0.12
Block*Gas*Light	3	0.48

Original in colour

(a)



(b)

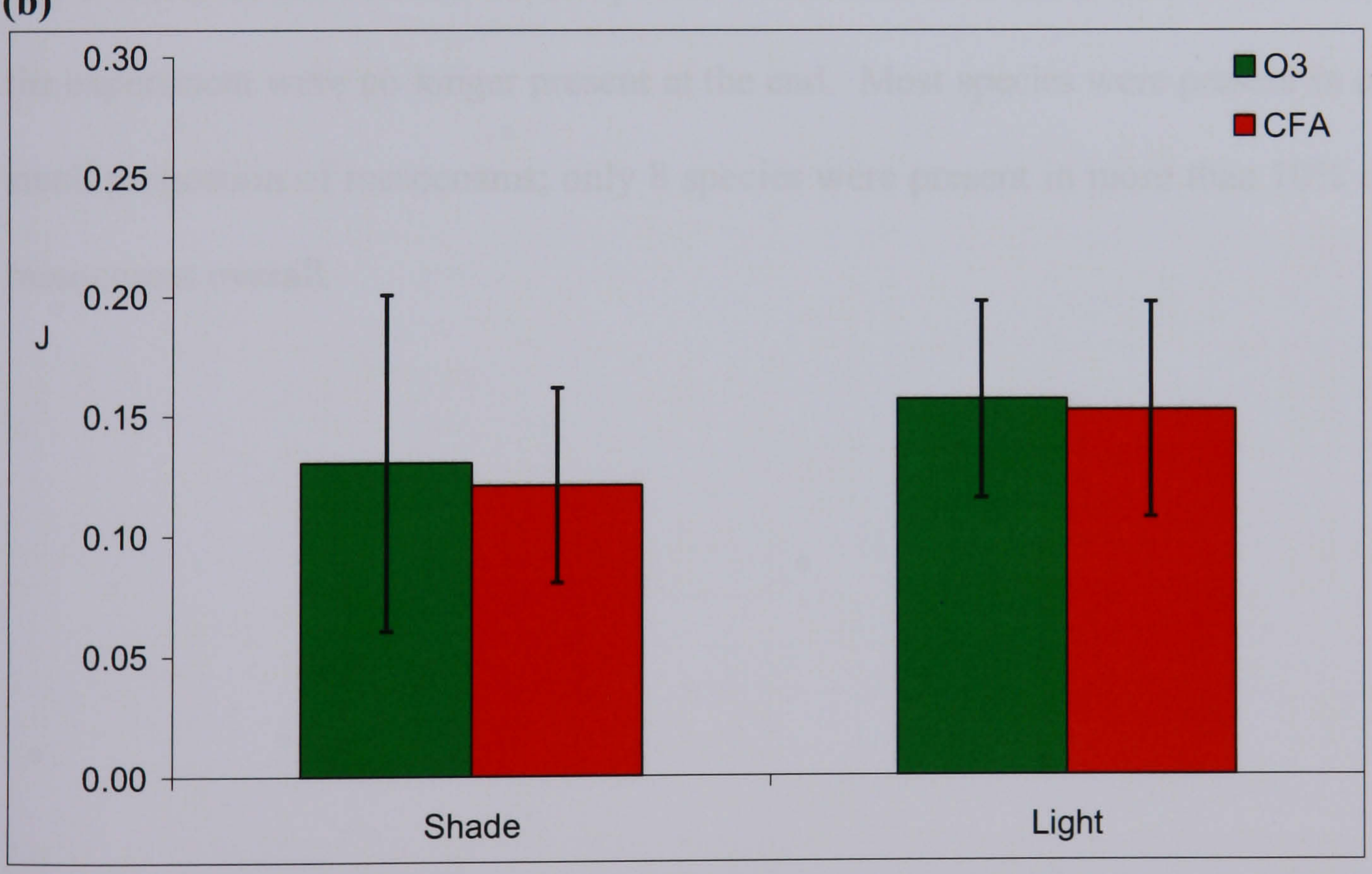


Fig 5.24
Mean (a) Shannon diversity index (H') and (b) Equitability (J) based on final live above-ground biomass values +/- 1s.e.

5.3.3 Experiment 3

5.3.3.1 Introduction

Mesocosms were placed in the fumigation chambers receiving filtered air while awaiting seedling emergence. After 3 weeks, seedlings emerged, and the ozone treatment started on 25/05/05. A total number of 30 species occurred in the 39 mesocosms. These species are listed in Table 5.25, ranked by their tolerance to shade as indicated by the Ellenberg Index for light with the frequencies of the species given for the end of the experiment.

By the end of the experiment, 5 species were present solely in the ozone treatments and 6 solely in the control. Three species which had been identified in the course of the experiment were no longer present at the end. Most species were present in only a small proportion of mesocosms; only 8 species were present in more than 10% of the mesocosms overall.

Original in colour

Table 5.25
Species present, ranked in order of shade tolerance according to the Ellenberg Light Index (L), showing and percentage frequency (fq) values for the end of the experiment.
NB. Percentage frequency indicates the % of mesocosms in which the species is present

L	Definition	Species	% Frequency				
			Total	Control	Ozone	Light	Shade
3	Shade Plants	*Mercuralis perrennis*	3	0	5	5	0
4	Between 3 and 5	*Veronica Montana*	3	5	0	0	5
		Geum urbanum	5	0	11	5	5
5	Semi-shade plants	*Potentilla sterelis*	0	0	0	0	0
		Silene doica	5	10	0	0	10
		Lysimachia nummularia	3	0	5	5	5
		Luzula pilosella	8	10	5	0	10
6	Between 5 and 7	*Ranunculus repens*	18	15	21	21	15
		Rubus fructicosus agg.	26	30	21	21	30
		Valeriania officinalis	5	5	5	0	10
		Viola rivinania	23	25	21	21	25
		Deschampsia caespitosa	13	5	21	11	15
		Chamerion agustifolium	0	0	0	0	0
		Glechoma hederacea	5	5	5	11	0
		Fragaria vesca	3	0	5	5	0
7	Light and partial shade plants	*Agrostis tenuis*	8	10	5	5	10
		Alchemilla glabra	5	10	0	5	5
		Cerastium fontanum	3	5	0	5	0
		Holcus lanatus	8	5	5	11	5
		Hypericum humifusum	26	25	26	32	20
		Luzula campestris	5	10	0	0	10
		Plantago lanceolata	10	10	11	21	0
		Poa annua	5	5	5	0	10
		Potentilla erecta	5	5	5	0	10
		Prunella vulgaris	5	5	5	5	5
		Rumex obtusifolium	0	0	0	0	0
		Sagina procumbens	38	30	47	47	30
		Stellaria media	5	0	11	5	5
		Juncus spp.	13	10	16	16	10
8	Light Loving plants	*Cardamine hirstuta*	3	5	0	0	5

5.3.3.2 Species Richness and Abundance

Table 5.26 gives the results of a repeated measures analysis for both species richness and abundance. Fig 5.25 (a) shows the mean species richness (r) and Fig 5.25 (b) shows the mean abundance for all four treatments. Both mean r and abundance have low mean scores for all treatments.

Table 5.26
Repeated measures analysis for Species Richness (r) and Abundance (A), **(a)** within subject and **(b)** between subject effects. :* = P<0.10; **= P<0.05, ***=P<0.01

(a)	r		A	
	d.f.	F	d.f.	F
Time	7.00	**22.86****	6.94	**7.00****
Time*Gas	7.00	1.42	6.94	1.01
Time*Light	7.00	**1.50****	6.94	**2.42***
Time*Gas*Light	7.00	**2.25***	6.94	1.46
Time*Block	21.00	**1.82***	20.82	**1.75***
Time*Block*Gas	21.00	**2.10****	20.82	**1.51***
Time*Block*Light	21.00	**2.49****	20.82	**2.27****
Time*Block*Gas*Light	21.00	1.05	20.82	0.80

(b)	d.f.	F	
		r	A
Block	3	1.81	1.07
Gas	1	0.03	0.04
Light	1	2.67	1.72
Block*Gas	3	1.73	0.49
Block*light	3	0.57	0.35
Gas*Light	1	0.19	0.74
Block*Gas*Light	3	1.05	0.46

There is a significant time effect on both species richness (P<0.01) and abundance (P<0.05), indicating large fluctuations over time as shown in Fig 5.25 (a) and (b). Generally mean values of both parameters increase over the time period in all treatments; although there are large differences between treatments over the course of

the experiment, by the end of the experiment all treatments have a similar mean value for both parameters.

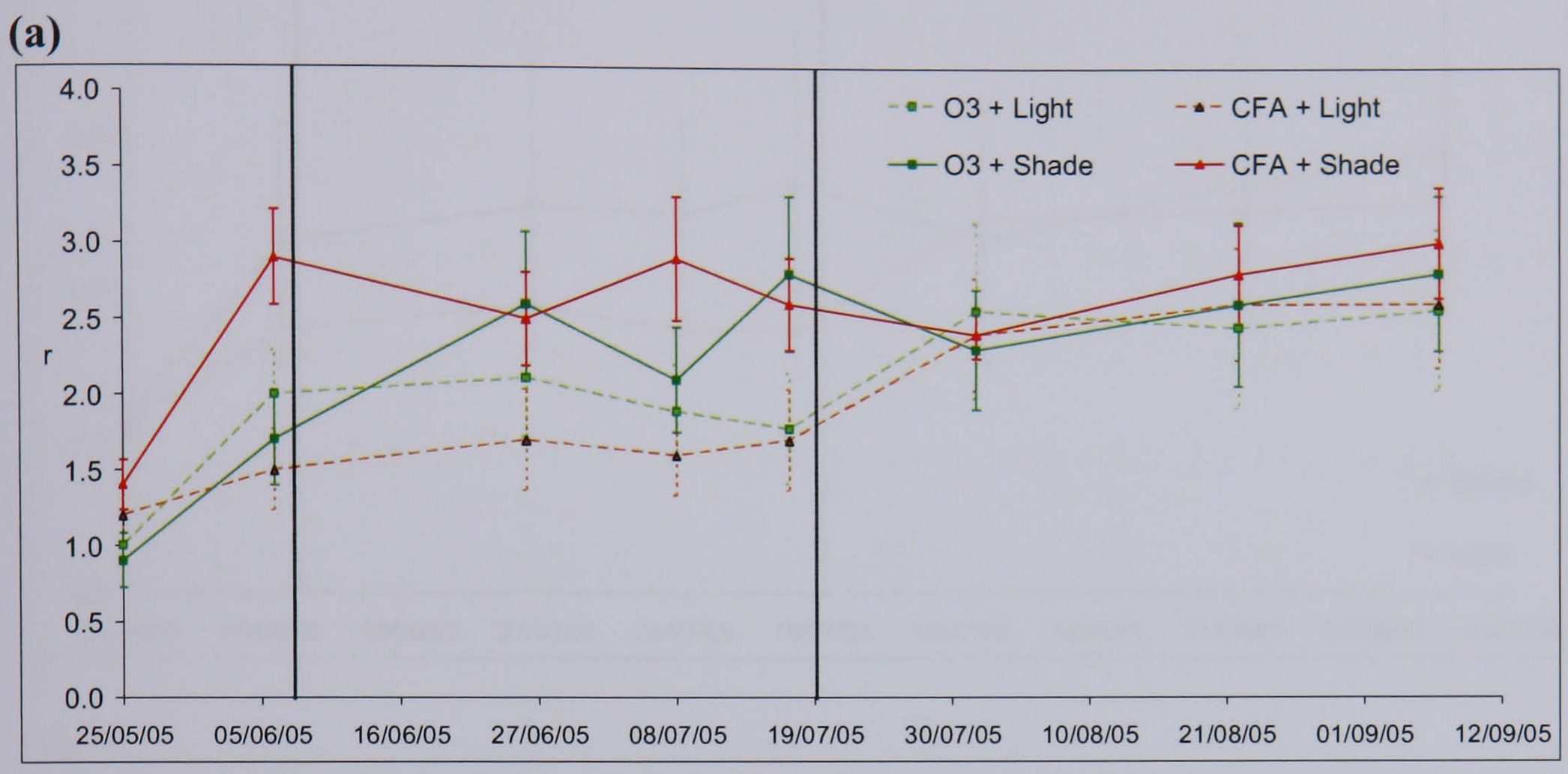
Species Richness

For r, the most important factor is the light treatment. There are two significant light interactions on species richness. Firstly there is a significant light/time interaction ($P<0.01$) and secondly there is a significant time/gas/light effect ($P<0.05$).

Fig 5.25 (a) shows that both shade treatments have larger fluctuations over time in mean r than the two light treatments. In these early stages of the experiment, the mean value of r in the shade treatments is higher than that in the light treatments; this effect is more clearly demonstrated in Fig 5.26 (a) which shows mean values of r in the light and. shade treatments over time. This effect is present from the earlier stages of the experiment and indeed is present before any shade treatments are started. There is some reduction in the mean value of r following each shade introduction. However there is no significant overall effect of light treatment on species richness.

The mean species richness increases following the second shade and by the end of the experiment the mean values for all treatments are similar. There are many block interactions that are significant for r; including time/block ($P<0.05$), time/block/gas ($P<0.01$) and time/block/light ($P<0.01$). Fig 5.26 (b) shows the mean r by block, and it is clear that each block has a different time course over the experiment. The fact that

there is no overall effect of light or gas treatment suggests that these variations between blocks in values of r over time confound the major treatment effects.



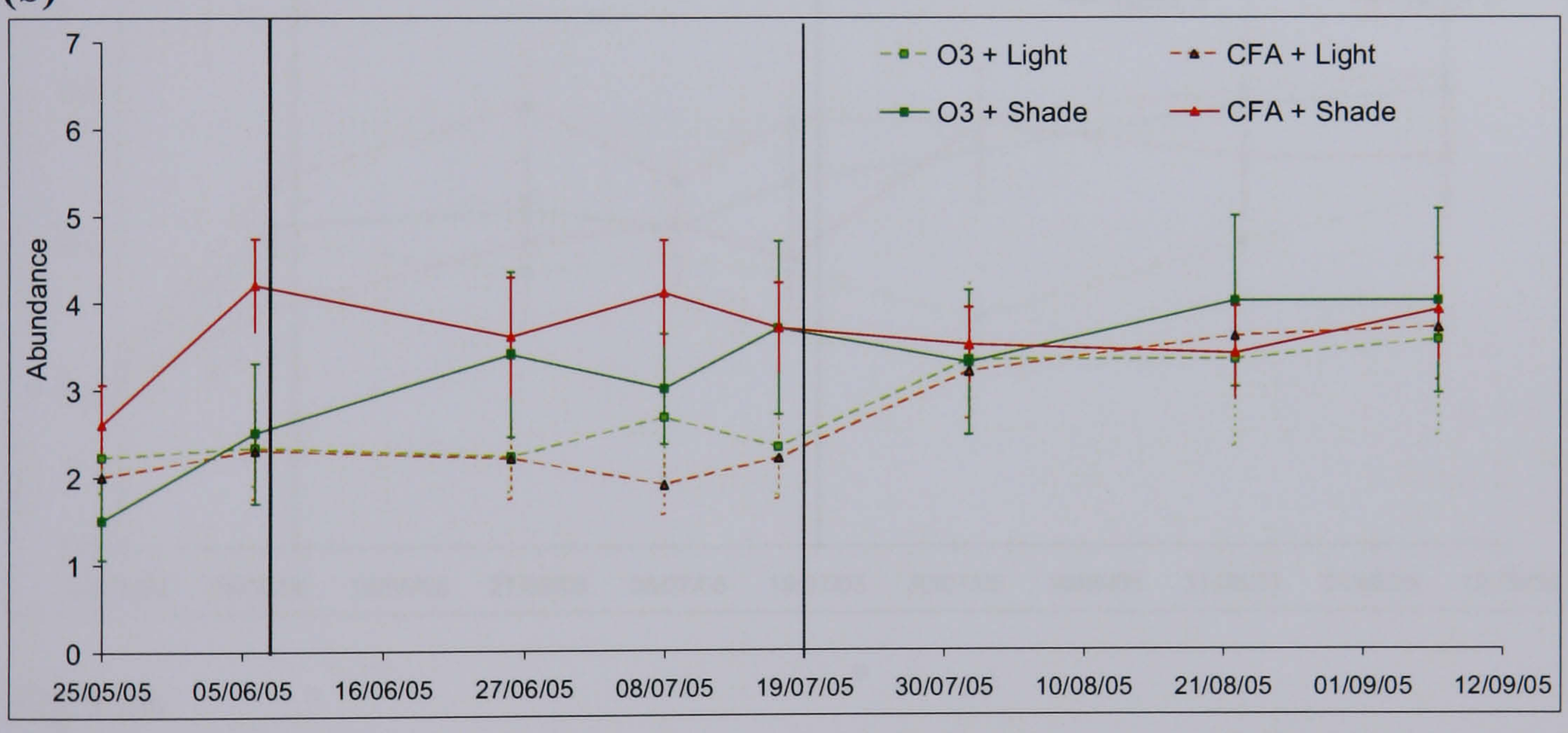
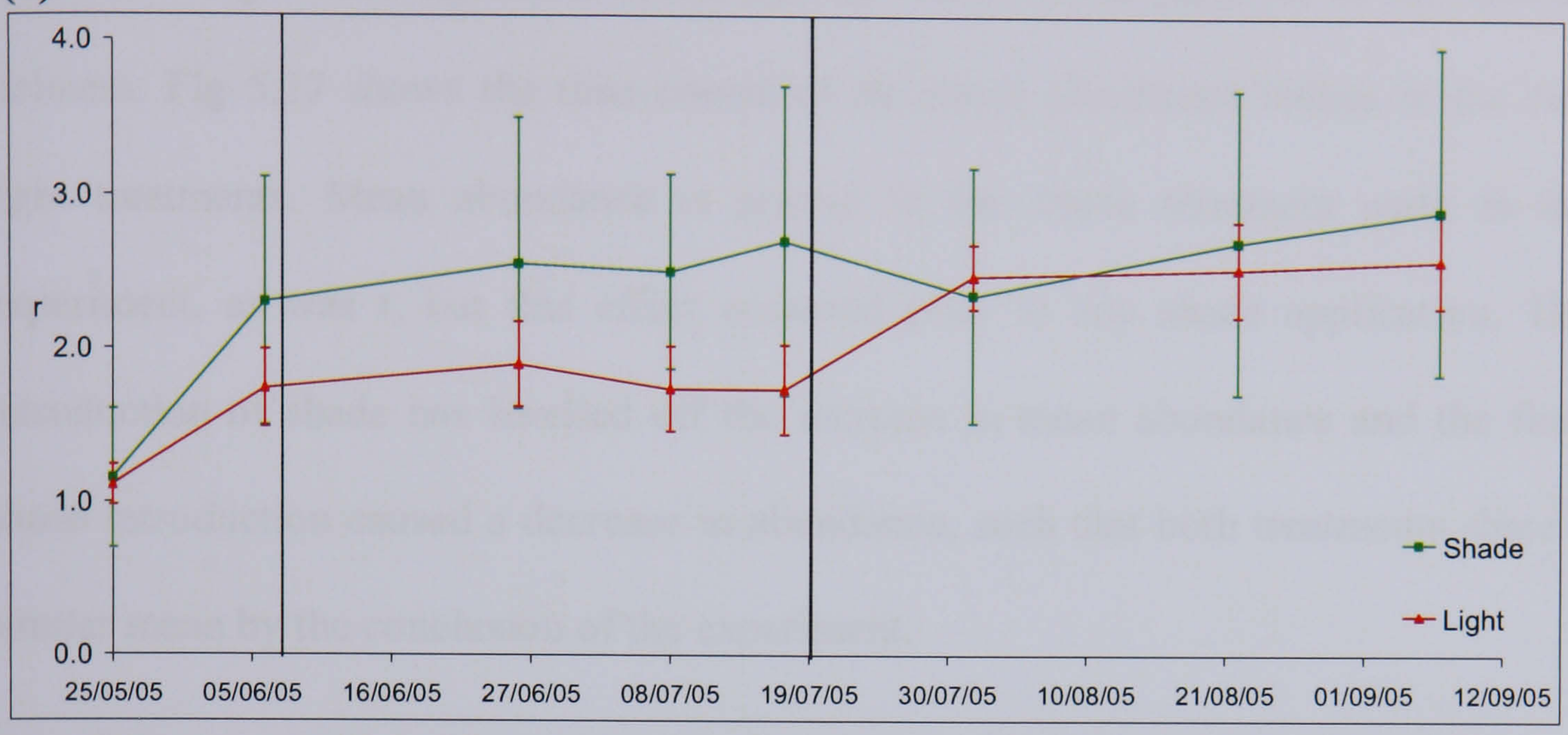


Fig 5.25
Mean **(a)** Species Richness (r) and **(b)** Abundance in the four treatments over time +/- 1s.e. First marker bar represent 1/3 reduction in light; Second represents 2/3 reduction in light.

(a)



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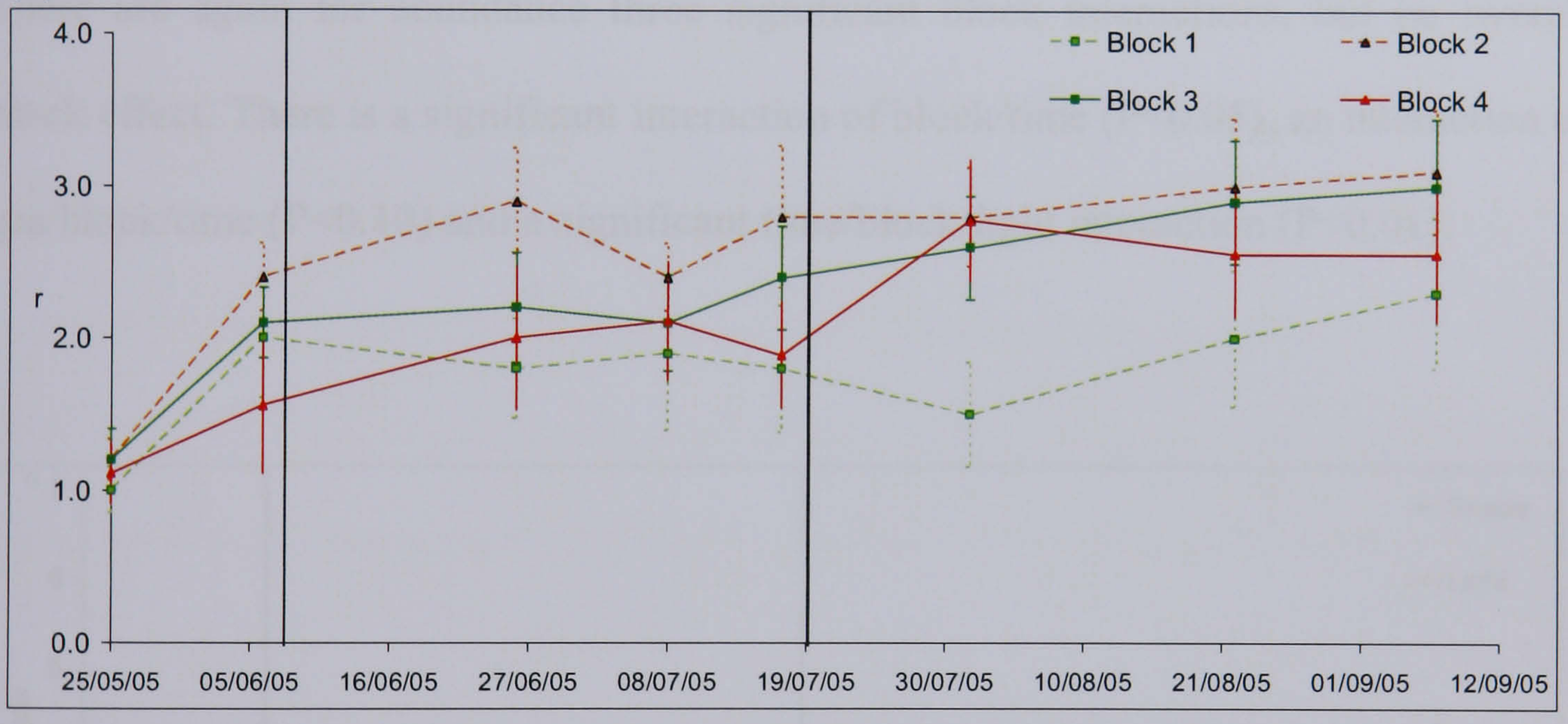


Fig 5.26
Mean Species Richness (r) as a function of: **(a)** light treatment and **(b)** block; over the experiment; +/- 1s.e.; First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light

Abundance

There is a significant light/time interaction ($P<0.05$) for abundance, as for species richness. Fig 5.27 shows the time-course of the mean abundance values in the two light treatments. Mean abundance is greater in the shade treatment early in the experiment, as was r, but this effect occurred prior to any shade application. The introduction of shade has levelled off the increase in mean abundance and the final shade introduction caused a decrease in abundance, such that both treatments share a similar mean by the conclusion of the experiment.

There are again for abundance three significant block interactions, but no overall block effect. There is a significant interaction of block/time ($P<0.05$), an interaction of gas/block/time ($P<0.10$) and a significant time/block/light interaction ($P<0.01$).

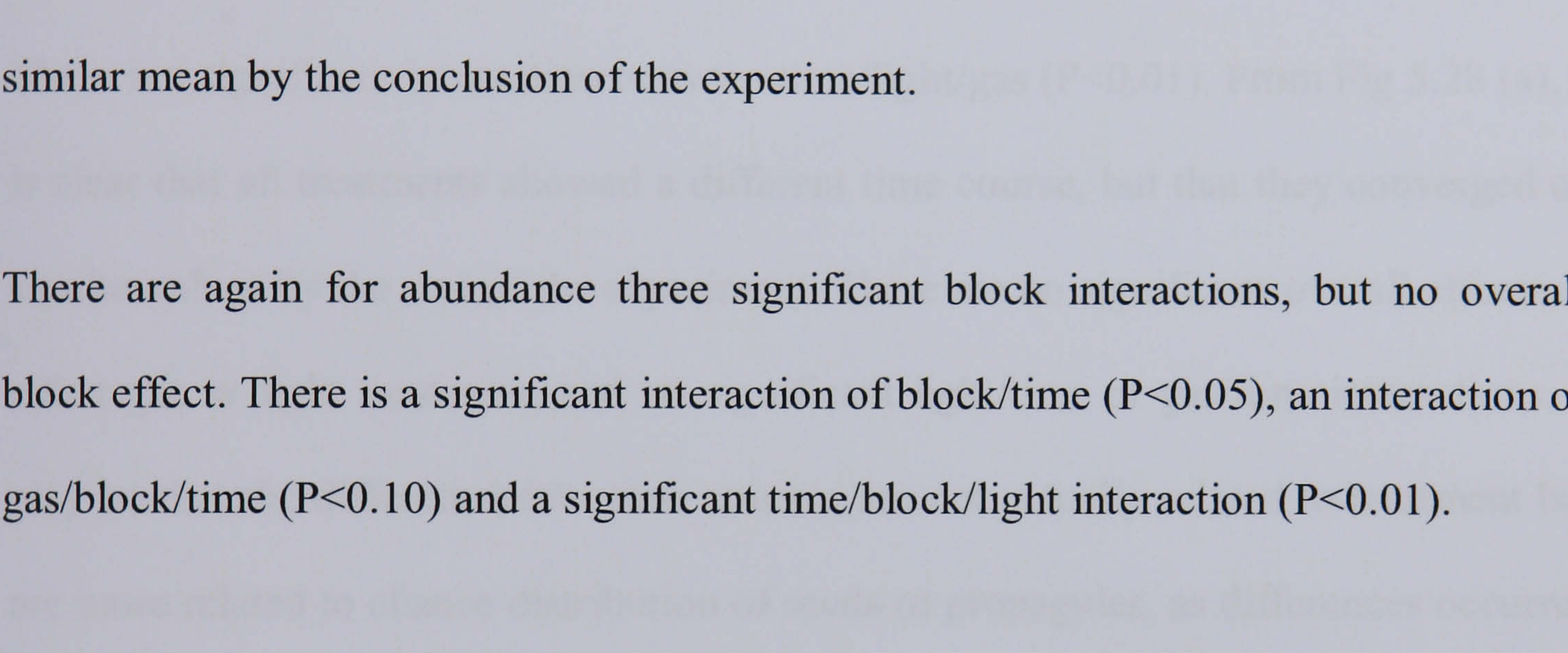


Fig 5.27
Mean Abundance over time in the two light treatments +/- 1s.e.: first marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light

5.3.3.3 Shannon diversity index (H') and Equitability (J)

Table 5.27 gives the results of the repeated measure analysis for H' and J. The mean values for all four treatments over the entire time period are shown in Fig 5.28 (a) Shannon Diversity and (b) equitability. There is a significant effect of time for both H' ($P<0.01$) and J ($P<0.01$).

Shannon diversity index

There is a significant interaction between time/light/gas ($P<0.01$). From Fig 5.28 (a), it is clear that all treatments showed a different time course, but that they converged on similar values by the end of the experiment. There are no significant overall effects of either gas or light treatment, and no significant light/time or gas/time interactions, it may be that the different initial time courses are not actually related to treatment but are more related to chance distribution of seeds or propagules, as differences occurred at the start of the fumigation and were lost as treatments progressed. There are two significant block interactions: time/block/gas ($P<0.01$) and time/block/light ($P<0.05$).

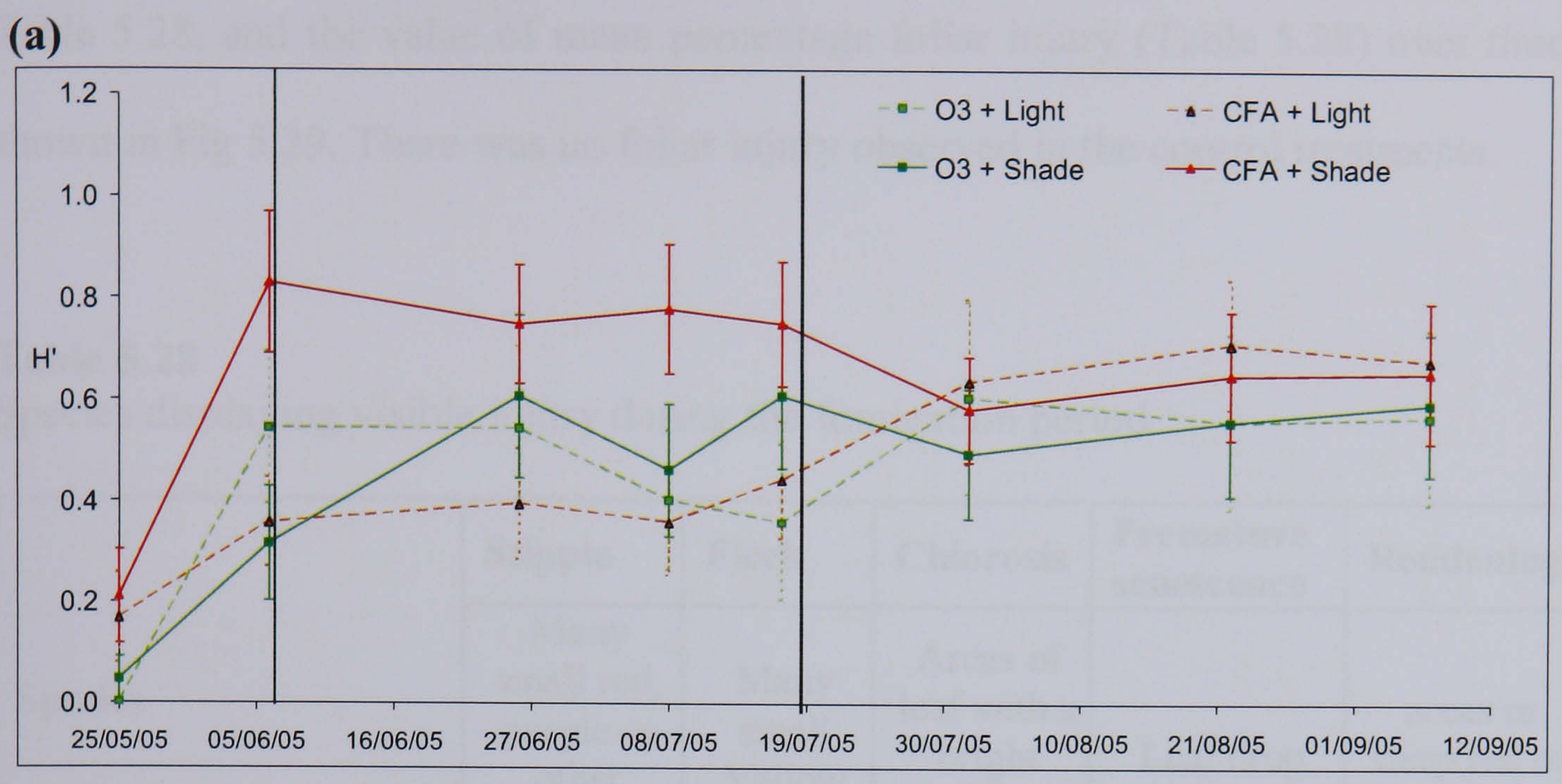
Equitability

As with species diversity, there is a significant interaction of time/gas/light ($P<0.01$), for equitability, but not of gas/time or light/time. The time course is also similar to that of species diversity, with the shade treatments showing a different response over the initial part of the experiment, but converging on a similar value towards the end. There is also a significant time/block/gas interaction ($P<0.01$).

Table 5.27
Repeated measures analysis for Shannon diversity index (H') and Equitability (J) **(a)** within subject and **(b)** between subject effects:
* = P<0.10; **= P<0.05, ***=P<0.01

(a)	H'		J	
	d.f.	F	d.f.	F
Time	7	**17.54*** **	7	**13.78*** **
Time*Gas	7	0.64	7	0.39
Time*Light	7	3.59	7	1.26
Time*Gas*Light	7	**2.91*** **	21	**2.52*** **
Time*Block	21	1.09	7	1.11
Time*Block*Gas	21	**2.51*** **	21	**2.02*** **
Time*Block*Gas*Light	21	0.49	21	0.54
Time*block*Light	21	**1.88** **	21	1.39

(b)	d.f.	F-values	
		H'	J
Block	3	0.59	0.77
Gas	1	0.75	2.14
Light	1	0.97	0.78
Block*Gas	3	0.75	0.96
Block*light	3	0.82	1.48
Gas*Light	1	0.26	0.01
Block*Gas*Light	3	0.50	1.08



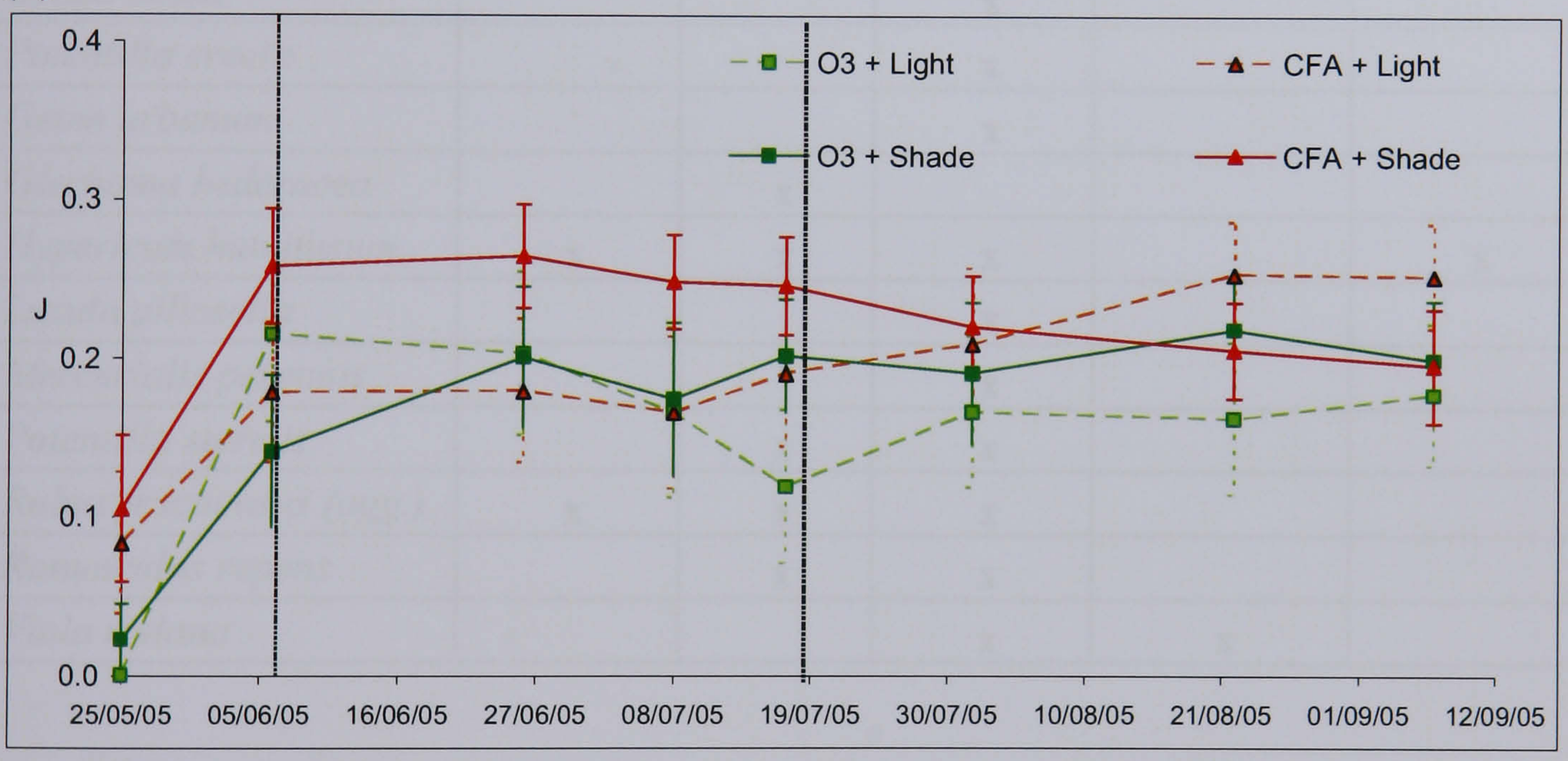


Fig 5.28
(a) Mean values of H' and **(b)** J over the course of the experiment +/- 1s.e. The first marker denotes 33% reduction in light; the second marker denotes 66% reduction

5.3.3.4 Foliar Injury

Table 5.28 gives a brief summary of the types of visible foliar injury which were observed over the time course of the experiment, and which species exhibited them.

Table 5.29 gives the results of repeated measures analysis for foliar injury as per Table 5.28, and the value of mean percentage foliar injury (Table 5.28) over time is shown in Fig 5.29. There was no foliar injury observed in the control treatments.

Table 5.28
Species displaying visible injury during the fumigation period

Species	**Stipple**	**Fleck**	**Chlorosis**	**Premature senescence**	**Reddening**
	Many small red, purple or other pigmented spots	Many small Yellow Spots	Areas of leaf with a bright yellow colour	Leaf drop	areas or deep red or purple
Urtica doica			**x**		
Potentilla erecta			**x**		
Geum urbanum			**x**		
Glechoma hederacea		**x**			
Hypericum humifusum	**x**	**x**	**x**		**x**
Luzula pillosella			**x**		
Mercurialis perennis			**x**		
Potentilla sterelis		**x**	**x**		
Rubus fructicosus (agg.)	**x**	**x**	**x**		
Ranunculus repens		**x**	**x**		
Viola riviana			**x**	**x**	

There is a significant effect of time ($P<0.01$) on visible injury, which increased early in the experiment, but not thereafter (Fig. 5.29). There is also a significant interaction of time/gas treatment ($P<0.01$) and a significant overall effect of gas treatment ($P<0.01$). Since there was no type of foliar injury observed in the control treatments it is clear that the ozone is linked to the occurrence and quantity of the different injury symptoms. Although ozone injury appeared to be lower in the shade treatment (cf. Fig. 5.29) this effect was not significant.

Table 5.29
Results of a repeated measures analysis For Percentage Visible Foliar Injury **(a)** within subject and **(b)** between subject effects: * = P<0.10, ** = P<0.05; *** = P<0.01

(a)	d.f.	F
Time	7	**2.66****
Time*Gas	7	**2.66****
Time*Light	7	0.51
Time*Gas*Light	7	0.51
Time*Block	21	**1.90****
Time*Block*Gas	21	**1.90****
Time*block*Light	21	**1.70****
Time*Block*Gas*Light	21	**1.70****

(b)	d.f.	F
Block	3	1.69
Gas	1	**17.09*****
Light	1	0.09
Block*Gas	3	1.69
Block*light	3	0.31
Gas*Light	1	0.09
Block*Gas*Light	3	0.31

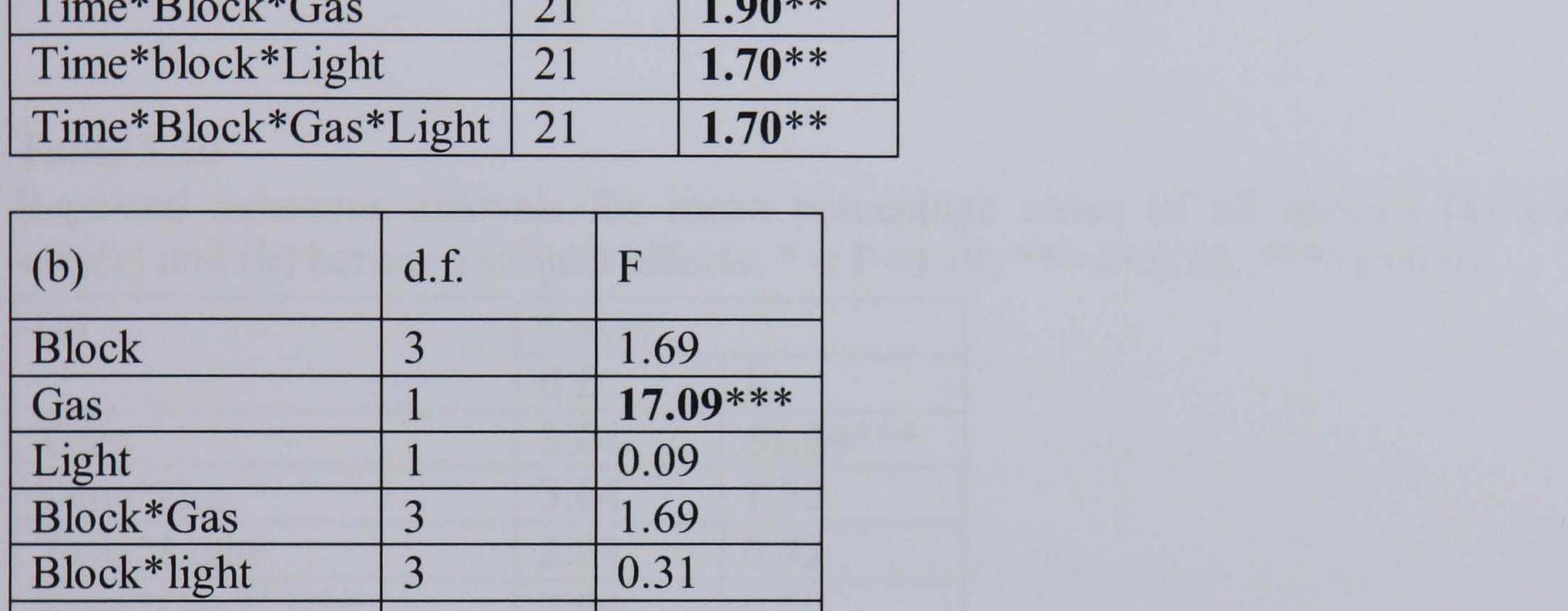


Fig 5.29
Mean percentage visible foliar injury by treatment over the course of the experiment (as described in Table 5.28), +/- 1s.e. First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light.

5.3.3.5 Percentage Cover

Table 5.30 gives the results of a repeated measures analysis for total percentage cover of all species, and the time-course of mean values in each treatment are illustrated in Fig 5.30. There is a significant time effect for percentage cover ($P<0.01$), due to a gradual increase in mean cover over time (Fig. 5.30). There are no significant effects of treatments and their interactions, and percentage cover seems to have not been impacted by reductions in light levels.

Table 5.30
Repeated measures analysis for mean percentage cover of all species **(a)** within subject and **(b)** between subject effects: * = P<0.10; **= P<0.05, ***=P<0.01

(a)	Cover	
	d.f.	F
Time	3.04	**51.59*** **
Time*Gas	3.04	1.35
Time*Light	3.04	0.42
Time*Gas*Light	3.04	0.50
Time*Block	9.11	1.69
Time*Block*Gas	9.11	1.13
Time*block*Light	9.11	1.61
Time*Block*Gas*Light	9.11	1.00

(b)	d.f.	F
Block	3	**2.48***
Gas	1	0.19
Light	1	0.31
Block*Gas	3	0.76
Block*light	3	0.25
Gas*Light	1	0.02
Block*Gas*Light	3	0.22

Original in colour

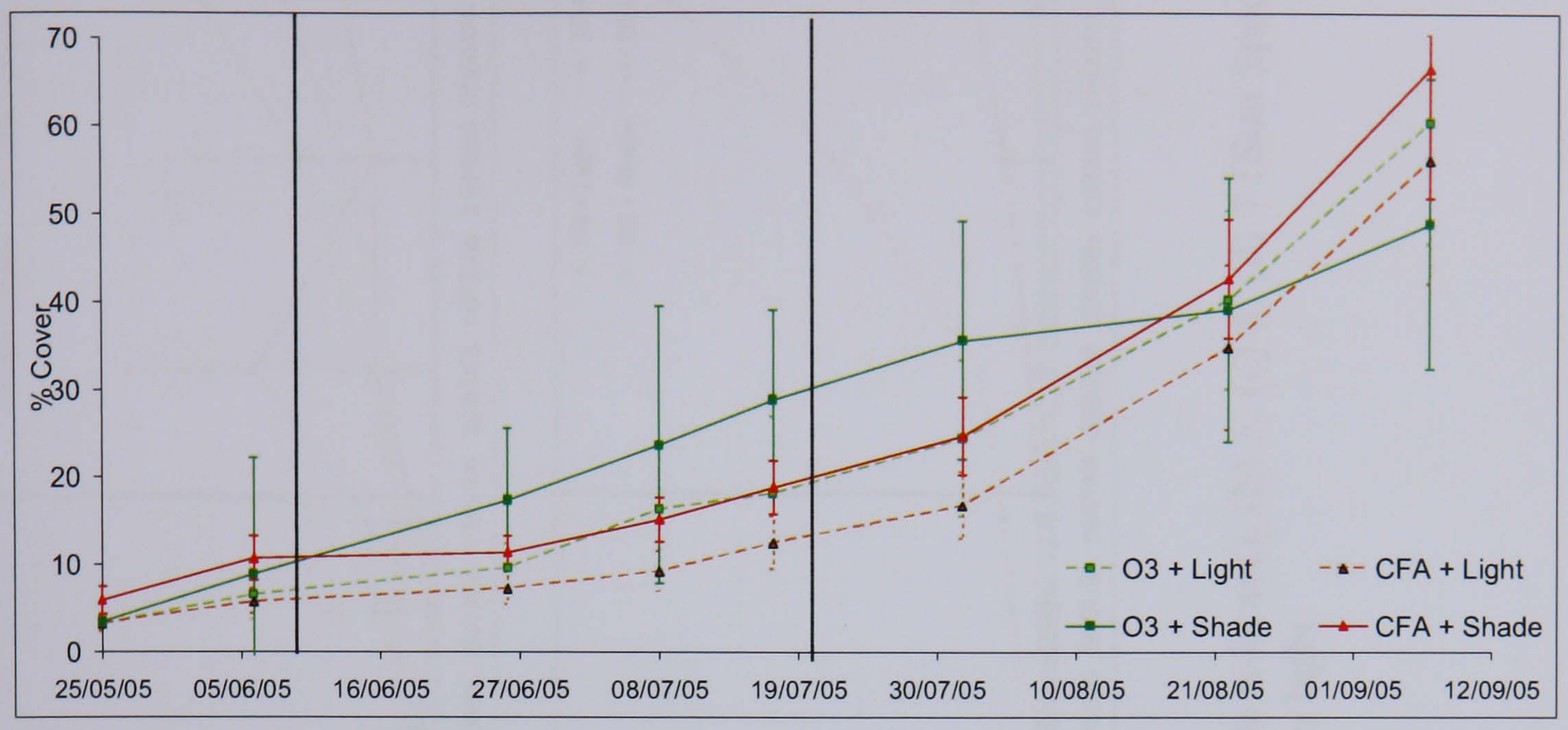


Fig 5.30
Mean percentage cover of all species over time +/- 1s.e.; First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light

5.3.3.6 Percentage Cover by Ellenberg Index

Table 5.31 gives the results of repeated measures analysis for percentage cover for different groups of species, based on their tolerance for shade as indicated by the Ellenberg Index for light (Table 5.2). Fig 5.31 (a-d) shows the time course of mean percentage cover within each group in the four treatments. Neither Group 3&4 or Group 5 showed any significant treatment effect, apart from a four-way interaction for Groups 3&4. This may reflect the very low overall cover in these groups. Therefore, detailed analysis was focussed on the results for Group 6 and Group 7.

Group 6

There is a large significant effect of time ($P<0.01$) on the mean percentage cover of Group 6. There is also an overall block effect ($P<0.10$) in Group 6. Fig 5.32 gives the mean percentage cover for Group 6 by block. From Fig 5.32, Block 3 is the most different showing a considerably higher mean over the time period than the other block. There is also a time/block interaction ($P<0.10$). The data in Fig. 5.31 (c)

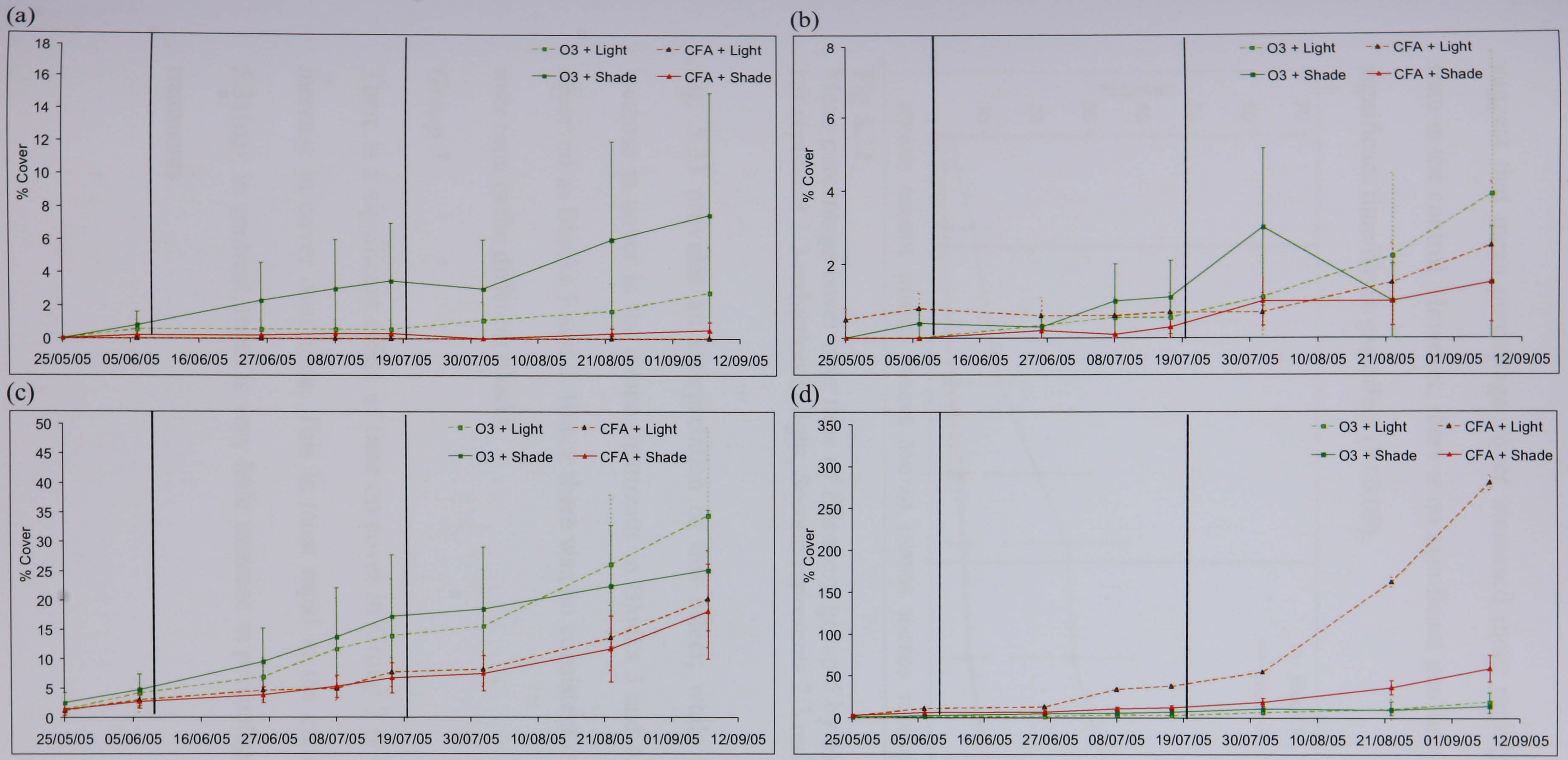


Fig 5.31
Mean percentage cover over time for four Ellenberg light categories; (a) 3 & 4 (most shade tolerant), (b) 5, (c) 6, (d) 7 (Sun Species), +/- 1s.e; First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light.

suggest that mean percentage cover increased more rapidly in the ozone treatment than in the control. However, there is no significant gas/time interaction, but there is a significant time/block/gas effect ($P<0.05$).

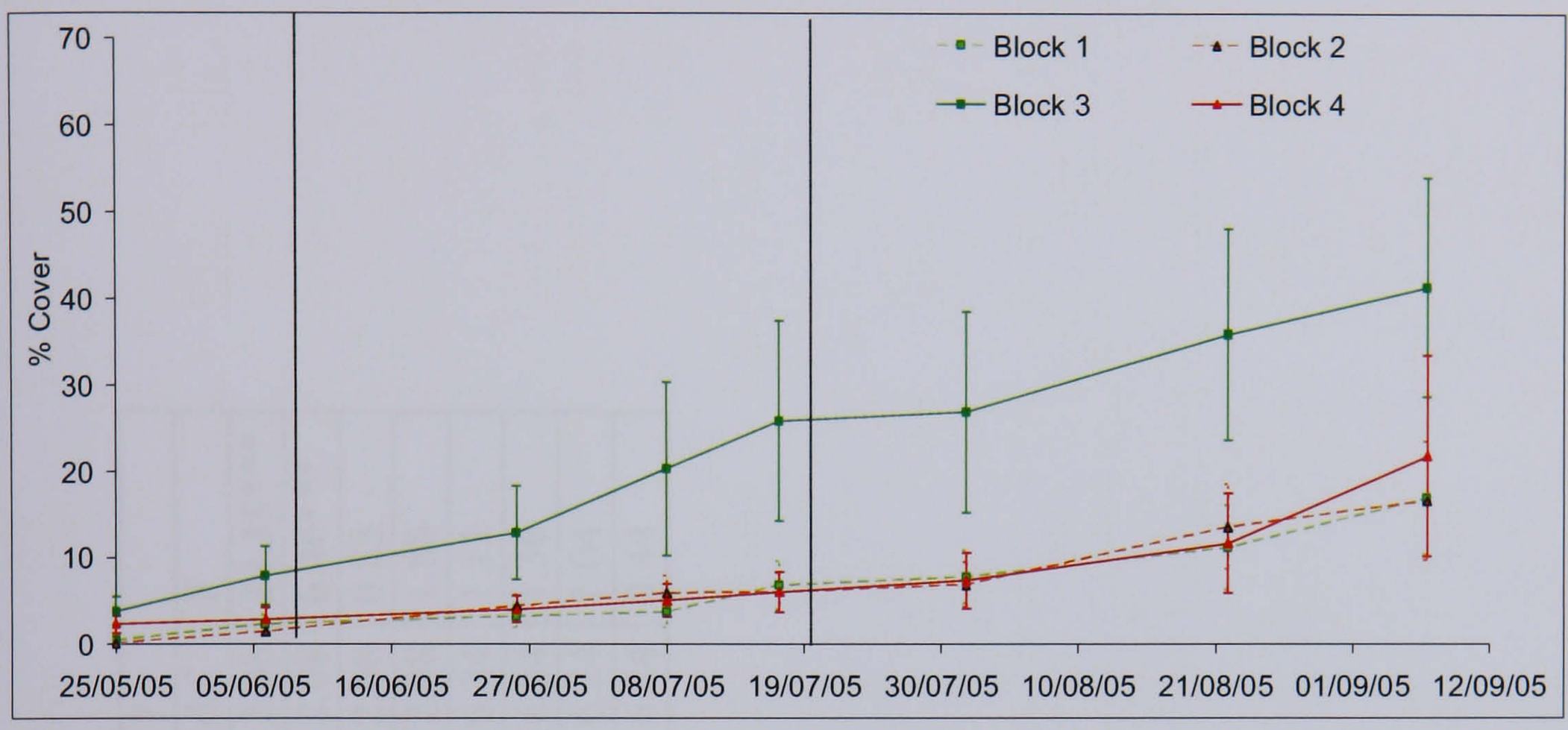


Fig 5.32
Mean percentage cover over time of Ellenberg group 6 by block +/1s.e. First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light.

Fig. 5.33 provides an interpretation of this effect; while there was a more rapid increase in cover in the ozone treatments in Blocks 3 and 4, the opposite effect was observed in Blocks 1 and 2. Hence there was no consistent effect of the gas treatment over time in the different blocks.

Group 7

There is a significant effect of time on cover in Group 7 ($P<0.01$); there is a general increase in cover over time. This is most rapid in the control light treatment (Fig. 5.31(d)); in contrast, there is very little increase in percentage cover in the two ozone treatments.

Table 5.31
Repeated measures analysis for percentage over by Ellenberg Index (a) within subject and (b) between subject effects:
* = P<0.10; **= P<0.05, ***=P<0.01

(a)	3 & 4		5		6		7	
	d.f.	F	d.f.	F	d.f.	F	d.f.	F
Time	1.75	**2.68****	2.20	2.30	3.21	**19.14*****	2.08	**24.35*****
Time*Gas	1.75	2.42	2.20	0.16	3.21	1.29	2.08	**6.46*****
Time*Light	1.75	0.85	10.66	0.49	3.21	0.82	2.08	0.83
Time*Gas*Light	1.73	0.71	5.31	0.25	3.21	0.26	2.08	1.88
Time*Block	3.00	1.34	16.78	0.78	9.64	**1.70***	6.24	1.11
Time*Block*Gas	5.26	1.43	8.82	0.41	9.64	**2.04****	6.24	1.36
Time*Block*Light	5.26	1.98	11.26	0.52	9.64	1.38	6.24	1.04
Time*Block*Gas*Light	5.26	**2.03***	22.25	1.03	9.64	0.27	6.24	0.44

(b)	d.f.	F			
		3&4	5	6	7
Block	3	1.35	0.55	**12.57***	1.18
Gas	1	2.34	0.03	1.80	**5.44****
Light	1	0.95	0.02	0.12	**3.71***
Block*Gas	3	1.48	0.48	1.93	0.53
Block*light	3	1.97	0.77	0.75	0.25
Gas*Light	1	0.74	0.04	0.00	2.45
Block*Gas*Light	3	2.04	0.87	0.02	0.42

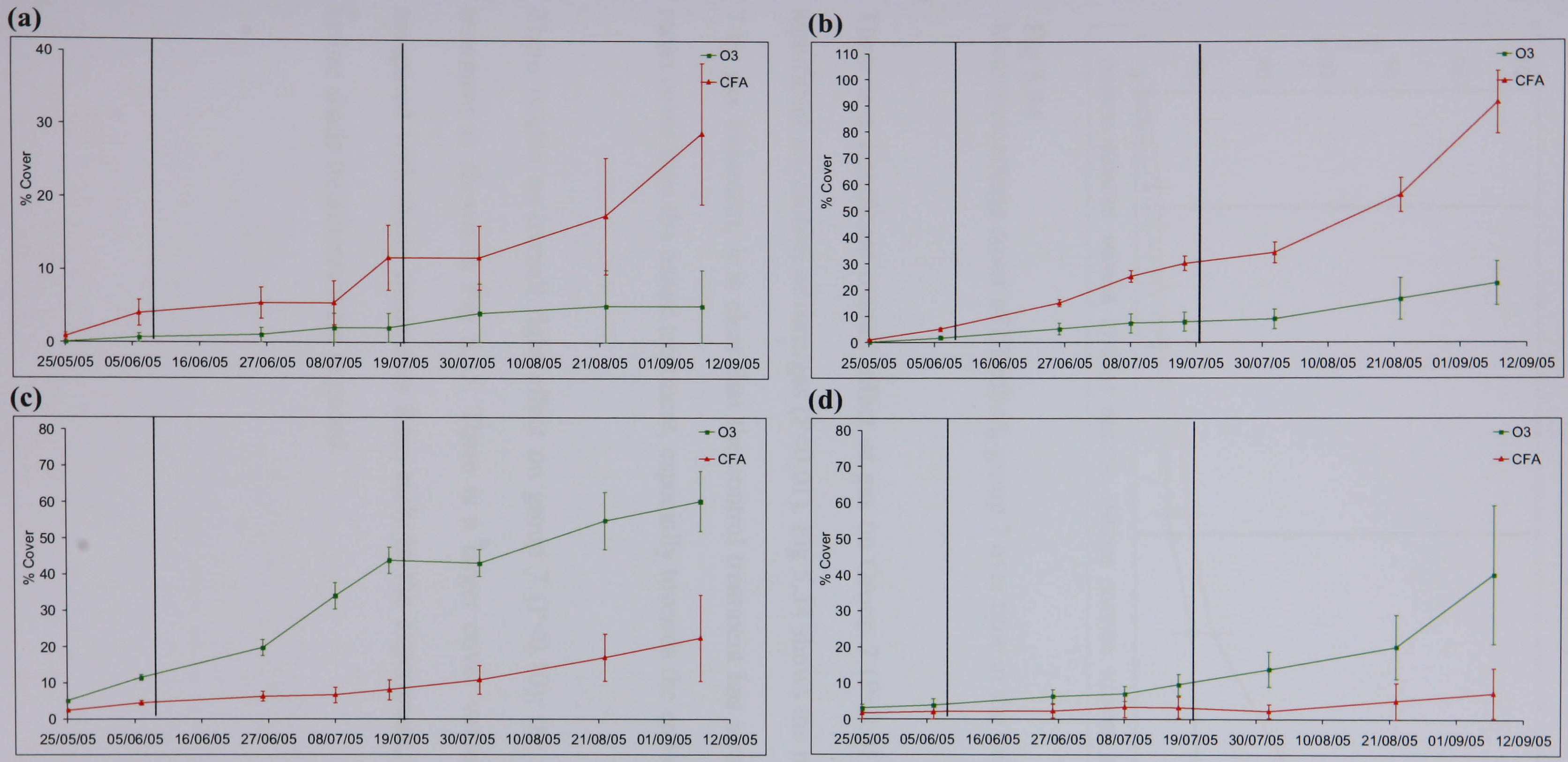


Fig 5.33
Mean percentage cover over time for Ellenberg Group 6 by Block; (a) Block 1, (b) Block 2, (c) Block 3, (d)Block 4, First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light.

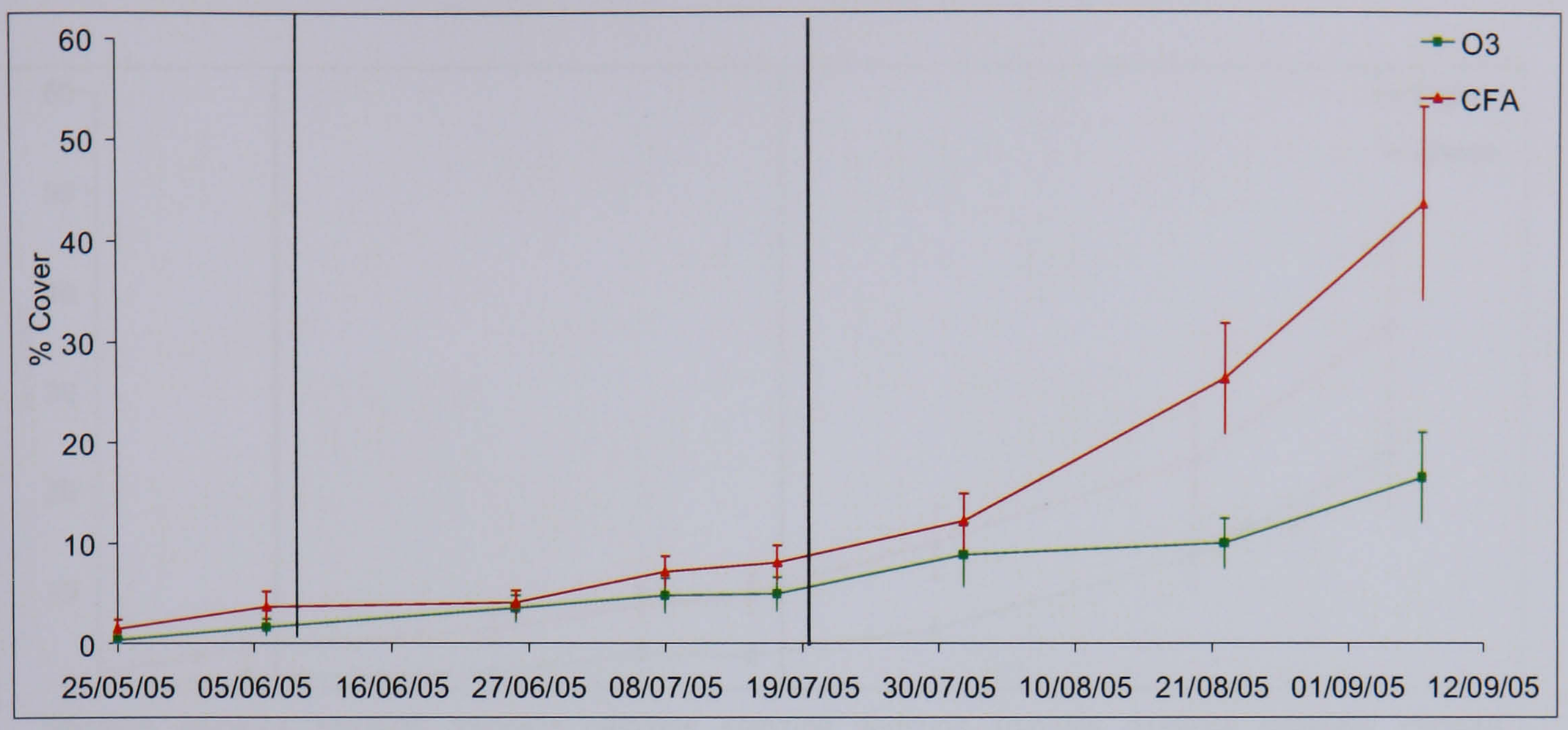


Fig 5.34
Mean percentage cover of Ellenberg group 7 over time in the two gas treatments.

There is a significant overall effect of gas on Group 7 ($P<0.01$), and there is also a significant interaction of time/gas ($P<0.01$). Fig 5.34 shows the mean cover for Group 7 by gas treatment; it is clear that the control treatment has a more rapid increase in mean cover than the ozone treatment, especially towards the end of the experiment.

There is also an overall light effect on group 7 ($P<0.10$); the mean cover by light treatment is shown in Fig 5.35. There is a larger cover value for light treatments compared with shade treatments from early in the experiment, including the period before shade treatments were imposed.

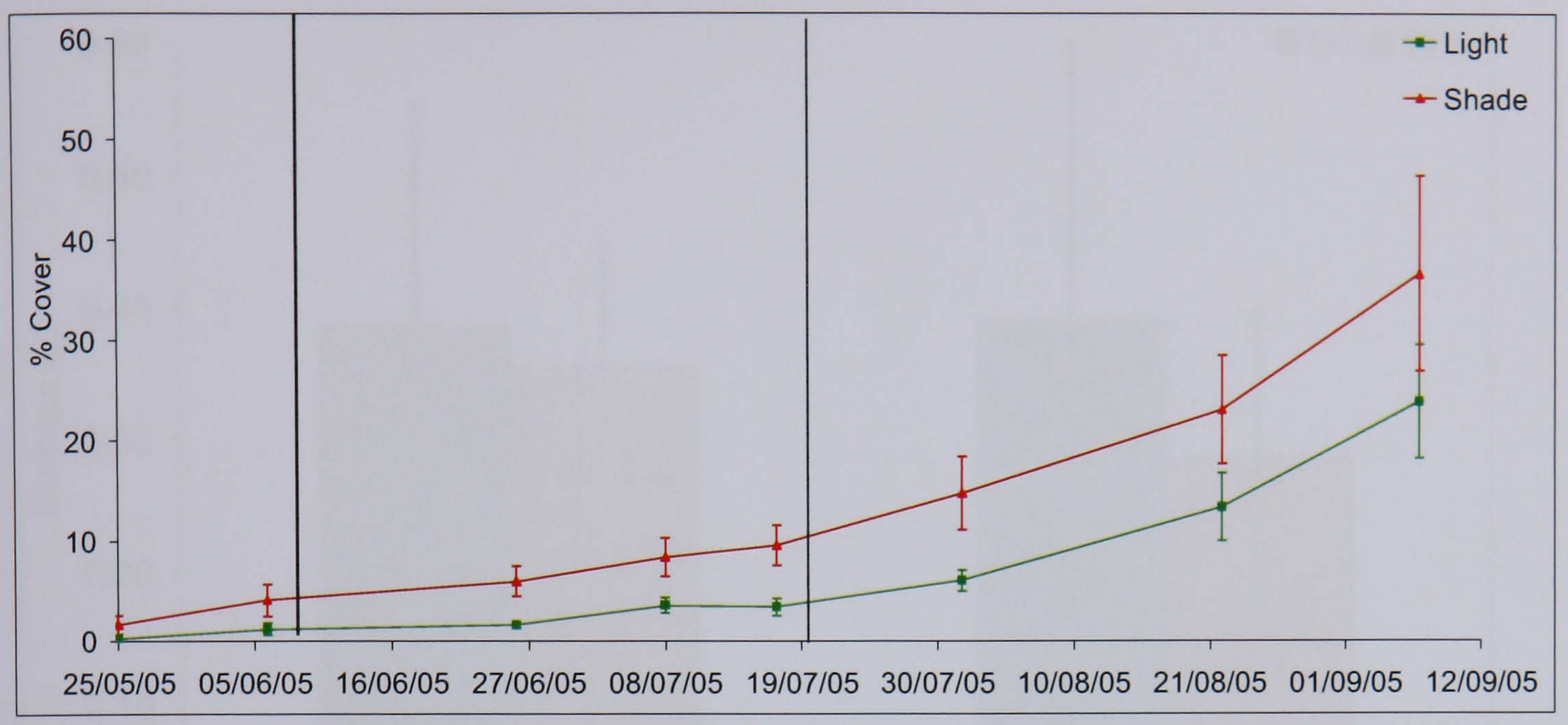


Fig 5.35
Mean percentage cover of Ellenberg Group 7 over time in the two light treatments. First marker bar represent 1/3 reduction in light, Second represents 2/3 reduction in light.

5.3.3.7 Biomass

Table 5.32 gives the results of an ANOVA analysis for live biomass and Fig 5.36 shows the mean total above-ground live biomass (g) in each treatment. There are no significant effects of block, gas or light or any significant interactions, although biomass is higher in the ozone treatment.

Table 5.32
Results of an ANOVA analysis for total above-ground live biomass at the end of the experiment.
* = P<0.10; **= P<0.05, ***=P<0.01

	Biomass	
	d.f.	F-Value
Block	3	1.47
Gas	1	0.03
Light	1	0.02
Block*Gas	3	0.78
Block*Light	3	0.24
Gas*Light	1	0.19
Block*Gas*Light	3	0.34

Original in colour

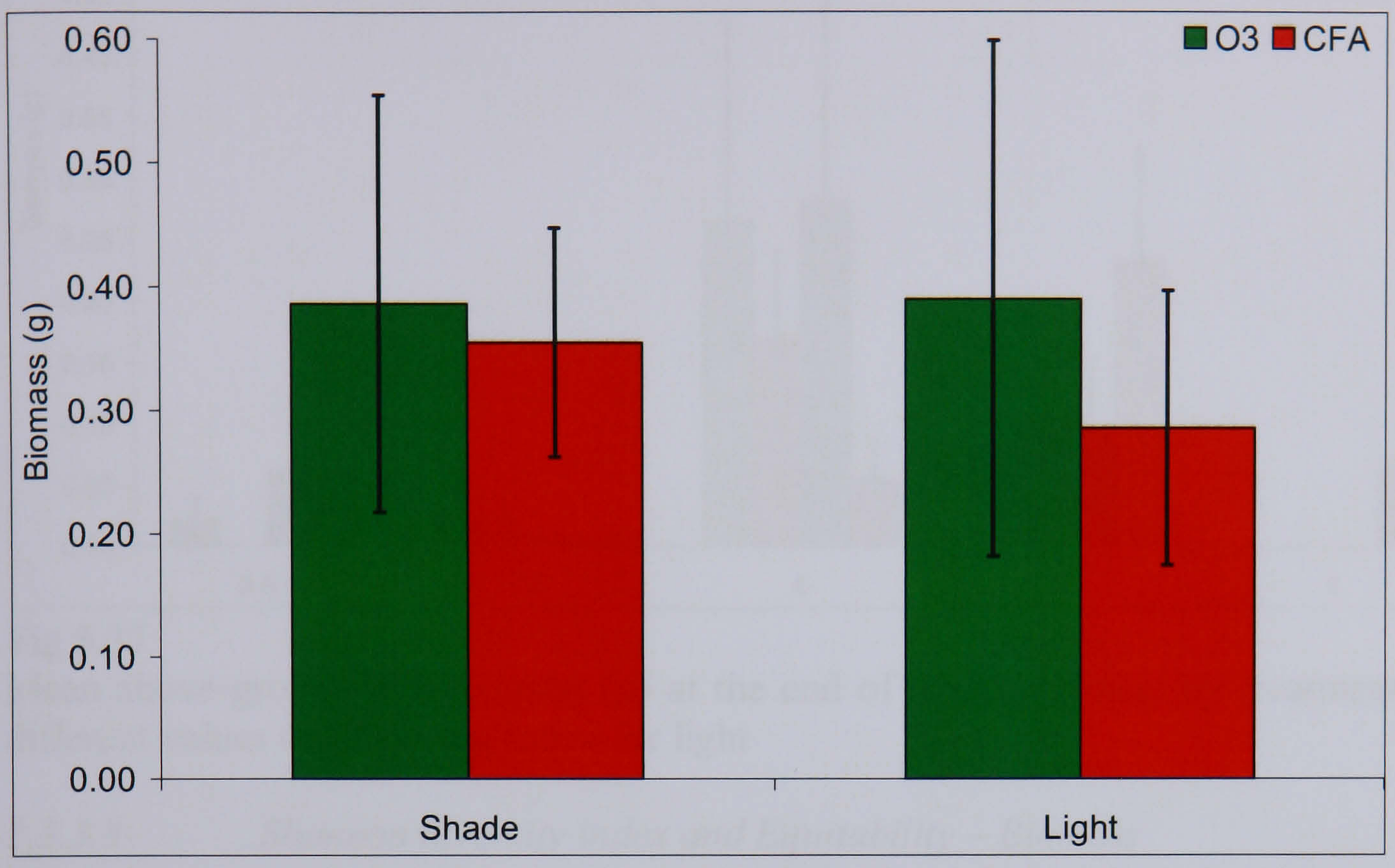


Fig 5.36
Mean total above-ground live biomass (g) by treatment at the end of the experiment +/- 1s.e.

Table 5.33
Results of an ANOVA analysis for above-ground live biomass ordered by Ellenberg index for light; * = P<0.10; **= P<0.05, ***=P<0.01

	d.f.	F-Values				
		3 & 4	5	6	7	8
Block	3	1.38	0.56	**2.37***	0.30	1.61
Gas	1	2.63	0.07	1.46	1.82	1.68
Light	1	0.74	0.53	0.44	0.65	1.68
Block*Gas	3	1.38	0.47	1.41	0.22	1.61
Block*Light	3	2.04	0.44	0.70	0.48	1.61
Gas*Light	1	0.74	0.11	0.29	0.47	1.68
Block*Gas*Light	3	2.04	0.84	0.26	0.31	1.61

Table 5.36 gives the results of an ANOVA analysis for live biomass ranked by the Ellenberg index for light. Fig 3.37 gives the mean live biomass per treatment also ranked by the index for light. There is a block effect for group 6 (P<0.10), but otherwise there are no significant effects or interactions for any group.

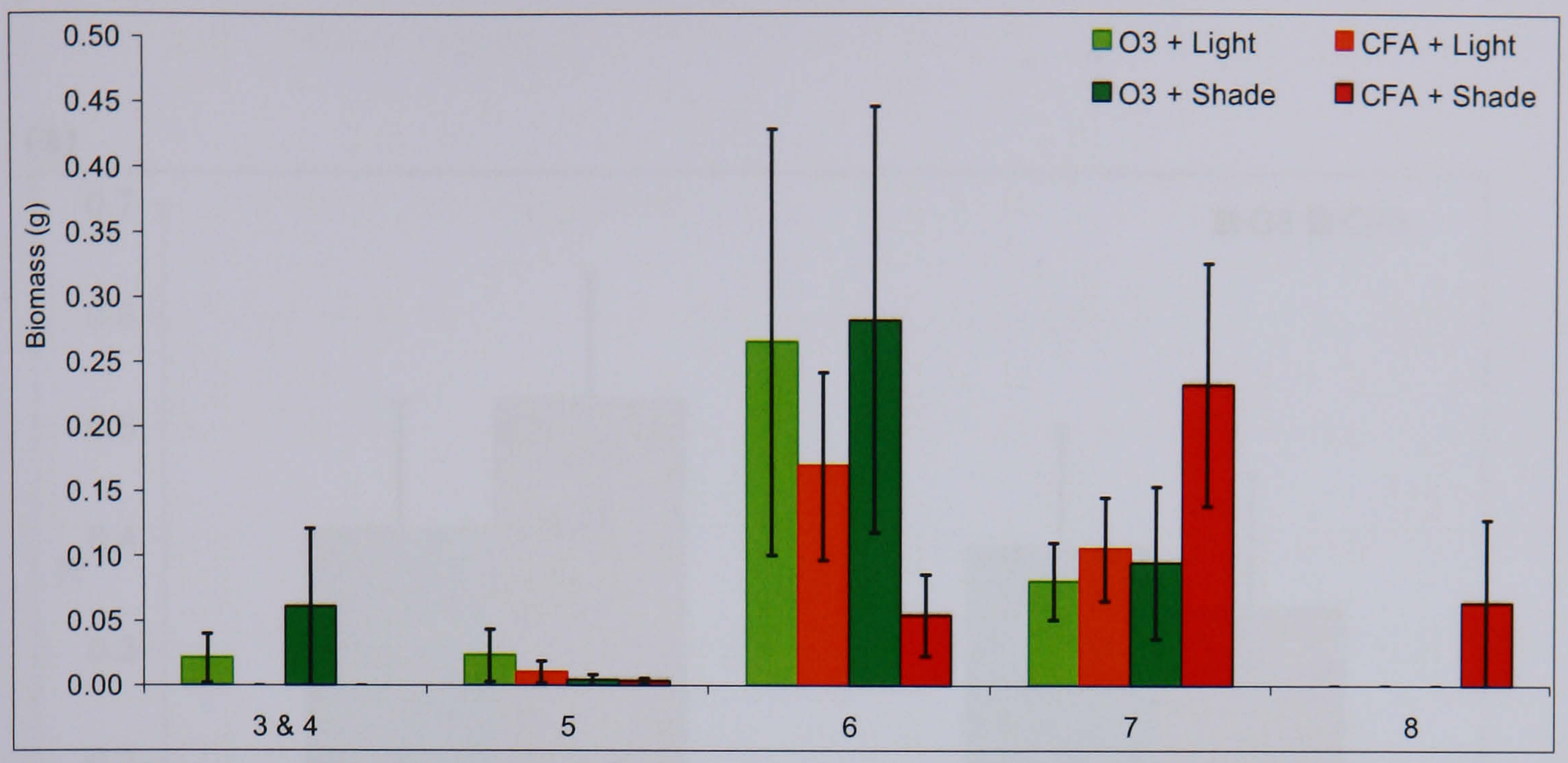


Fig 5.37
Mean above-ground live biomass (g) at the end of the experiment by treatment, for different values of Ellenberg Index for light

5.3.3.8 Shannon diversity index and Equitability – Biomass

Table 5.34 gives the results of an ANOVA analysis for Shannon diversity index and Equitability, based on the above-ground biomass data. Fig 5.38 shows the mean value of the indices by treatment. There is a block/gas interaction for Equitability (P<0.10), but here are no other significant effects or interactions for either index.

Table 5.34
Results of an ANOVA analysis for Shannon diversity index and Equitability based on final above-ground biomass.
* = P<0.10; **= P<0.05, ***=P<0.01

	d.f.	F	
		H'	J
Block	3	0.22	0.23
Gas	1	0.23	0.29
Light	1	0.78	0.69
Block*Gas	3	2.20	**2.79***
Block*Light	3	0.55	0.48
Gas*Light	1	1.00	2.12
Block*Gas*Light	3	0.13	0.06

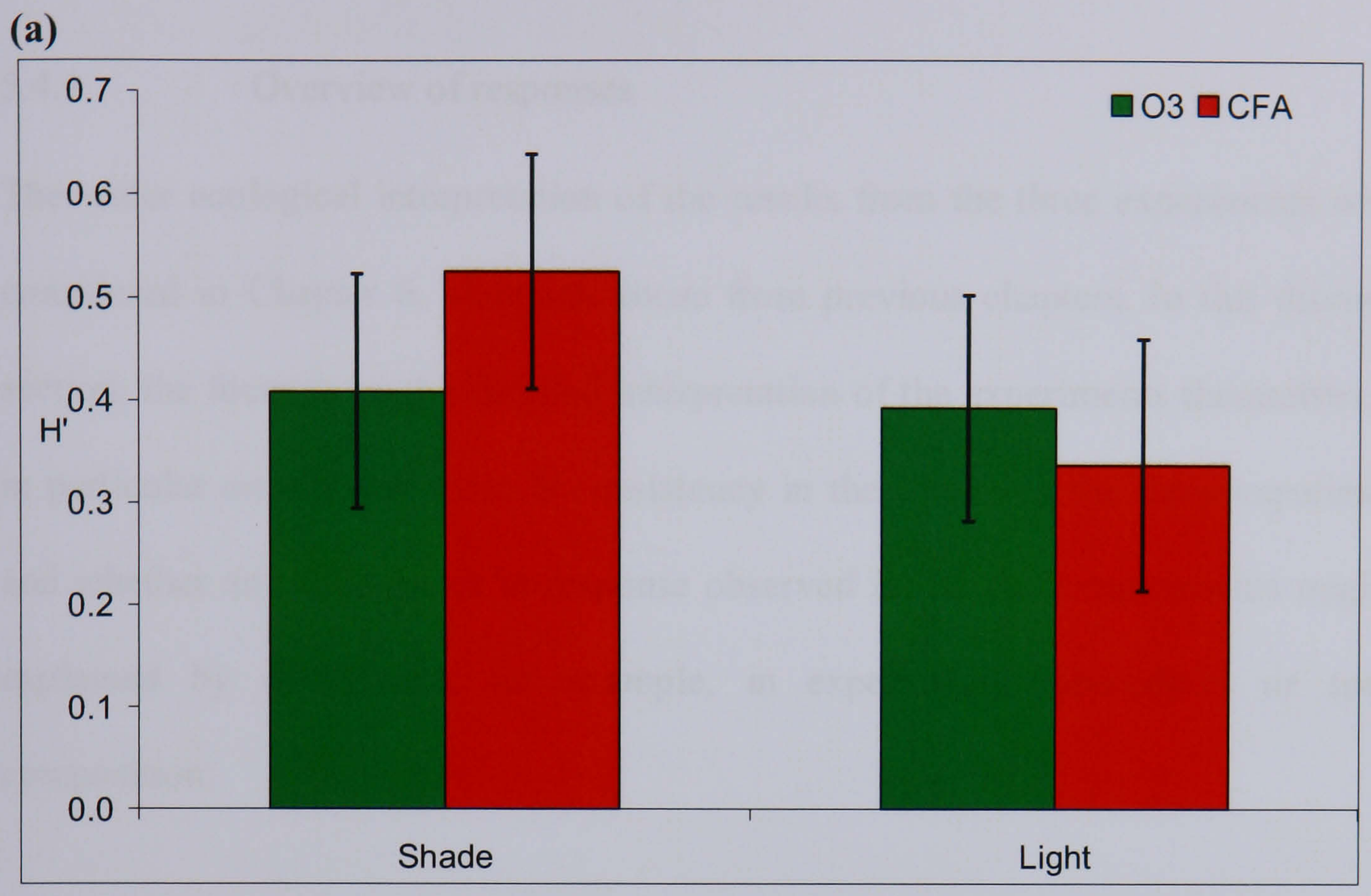


Fig 5.38
Mean (a) Shannon diversity index (H') and (b) Equitability (J), based on final biomass values

5.4. Discussion

5.4.1 Overview of responses

The wider ecological interpretation of the results from the three experiments will be considered in Chapter 6, alongside those from previous chapters. In this discussion section, the focus is on the detailed interpretation of the experiments themselves, and in particular on whether there is consistency in the results of the three experiments, and whether any differences in response observed in the three experiments might be explained by differences, for example, in experimental procedures or species composition.

Table 5.35 gives a summary of responses of the measured indices to ozone treatment in the three experiments. In broad terms, there were some consistent trends in the three experiments. In particular:-

- Cover and biomass were always reduced by ozone when significant effects were found;
- Shannon diversity index and equitability were always reduced when significant effects were found;
- Visible injury was always present and increased by ozone.

However, other effects were more variable. In particular:-

- The presence of significant effects on total biomass and cover differed between experiments;
- There were variable effects on species richness, diversity and abundance;

- There were variable results in terms of which group of species, based on Ellenberg light values, were affected.

Table 5.35
Summary of ANOVA results for all measured indices: ***a*** = Overall Ozone Effect, ***b*** = Ozone*Time Interaction. Arrows indicate direction in effect of ozone; ↑ = Increase, ↓ = Decrease, ~ = Varied Effect; an x indicates no data,

	Experiment					
	1		2		3	
	a	*b*	*a*	*b*	*a*	*b*
General Indices						
Species Richness	↑	ns	↓	↓	ns	ns
Abundance	↑	ns	↓	ns	ns	ns
Shannon diversity index (H')	ns	~	ns	↓	ns	ns
Equitability (J)	ns	~	ns	↓	ns	ns
Foliar Injury	↑	↑	↑	↑	↑	↑
Percentage Cover	ns	ns	↓	↓	ns	ns
Ellenberg Index Cover						
Groups 3 & 4	ns	ns	↓	↓	ns	ns
Group 5	ns	ns	ns	ns	ns	ns
Group 6	ns	ns	↓	ns	ns	ns
Group 7	ns	ns	ns	ns	↓	↓
Group 8	ns	ns	x	x	x	x
Biomass Indices						
Live Biomass	ns	x	↓	x	ns	x
Dead Biomass	ns	x	x	x	x	x
Shannon diversity index (H')	ns	x	↓	x	ns	x
Equitability (J)	↓	x	ns	x	ns	x
Ellenberg Biomass						
Groups 3 & 4	ns	x	ns	x	ns	x
Group 5	ns	x	ns	x	ns	x
Group 6	↓	x	ns	x	ns	x
Group 7	ns	x	↓	x	ns	x
Group 8	ns	x	x	x	ns	x

Hence, overall the results do suggest that ozone had a significant impact on the growth, diversity and composition of the mesocosms, but that its effects were very different in the different mesocosms. In order to assess why these variable responses were found, differences in the species present, growth patterns and other factors between the three experiments first need to be considered.

5.4.2 Effects of ozone on community structure

Table 5.36 lists the first 5 species with the greatest contribution towards mean biomass for all three experiments by treatment, each species is given a rank from 1-5; 1 indicating the most dominant in biomass and 5 the least. Table 5.37 gives a summary of the key indices at the conclusion of the experiment: mean biomass per area (10^{-3} g cm^{-2}); mean percentage cover; and number of species; biomass per unit area (10^{-3} g cm^{-2}) in order of Ellenberg light groupings are given in Table 5.38.

The mesocosms were most similar in terms of floristic components in Experiments 1 and 3 although they differed in mean cover and mean biomass of the most abundant species. The soil for these experiments came from the same area of woodland and the mesocosms were mainly comprised of species which are common in both open habitats and woodland rides. In contrast, the soil for Experiment 2 came from a coppice plot and represented a late successional woodland community, distinguished by an almost complete absence of any open habitat species.

Table 5.36
The top five largest contributing species to mean biomass per treatment for all three experiments.
% = percentage contribution to mean biomass, *r = biomass ranking between 1 and 5, 1 = largest contribution to biomass.*

Ellenberg Light Index	Species	Experiment 1				Experiment 2								Experiment 3							
		CFA+ shade		O3 + shade		CFA+ light		O3+ light		CFA+ shade		O3+ shade		CFA+ light		O3+ light		CFA+ shade		O3+ shade	
		%	*r*	%	*r*	%	*r*	%	*r*	%	*r*	%	*r*	%	*r*	%	*r*	%	*r*	%	*r*
3	*Fagus sylvatica*											22	*4*								
3	*Mercuralis perrenis*					19	*3*	16	*4*	20	*1*							10	*5*		
4	*Geum urbanum*					25	*2*									6	*4*			11	*4*
5	*Potentilla sterelis*							20	*2*												
5	*Schrophularia nodosa*	8	*4*																		
6	*Aegopodium podagraria*									14	*5*	17	*3*								
6	*Fragaria vesca*							23	*1*	18	*3*										
6	*Glechoma hederacea*											59	*1*	14	*4*						
6	*Holcus mollis*	5	*5*																		
6	*Ranunculus repens*			3	*5*															7	*5*
6	*Rubus fructicosus*					10	*4*	19	*3*	16	*4*			24	*1*	62	*1*			37	*1*
6	*Valeriania officinalis*																				
6	*Viola riviania*					10	*5*			20	*1*	7	*5*	21	*2*					17	*2*
7	*Alchemilla glabra*																	11	*3*		
7	*Holcus lanatus*	13	*2*	6	*4*											8	*2*	27	*1*		
7	*Hypericum humifusum*					27	*1*	16	5			24	*2*								
7	*Luzula campestris*																	11	*4*		
7	*Plantago lanceolata*													15	*3*	5	*5*				
7	*Poa annua*			8	*3*									12	*5*						
7	*Potentilla erecta*																			13	*3*
7	*Sagina procumbens*															7	*3*				
7	*Stellaria media*	11	*3*	17	*2*																
8	*Cardamine hirstuta*	45	*1*	52	*1*													18	*2*		

Table 5.37
Summary of Key Indices at the end of the exposure period, for all three experiments per treatment; Mean (± 1s.e.) biomass per unit area (10^{-3} g $cm^{-2)}$; Mean (± 1s.e.) percentage cover and total number of species

Parameter	Treatment	Experiment 1	Experiment 2	Experiment 3
Mean biomass per unit area (10^{-3} g cm^{-2}) ± 1s.e.	O3 + Light	-	1.5 ± 0.73	3.5 ± 1.79
	CFA + Light	-	4.0 ± 1.08	1.4 ± 0.46
	O3 + Shade	8.8 ± 0.91	1.5 ± 0.66	2.2 ± 0.98
	CFA + Shade	11.3 ± 1.10	2.4 ± 0.77	1.8 ± 0.53
Mean percentage cover (cm^2) ± 1s.e.	O3 + Light	-	46 ± 17.2	60 ± 13.8
	CFA + Light	-	80 ± 21.6	56 ± 14.1
	O3 + Shade	205 ± 15.3	44 ± 9.1	49 ± 12.3
	CFA + Shade	206 ± 12.6	77 ± 18.0	67 ± 14.7
Number of Species	O3 + Light	-	7	14
	CFA + Light	-	7	14
	O3 + Shade	38	11	15
	CFA + Shade	25	9	15

A total of 47 species were represented in the 3 mesocosm experiments. The large majority of species were open-habitat/light loving species, commonly found in seed bank samples due to their ability for long-term seed dormancy. Only eight species were present in all three experiments: *M.perrennis; P.sterelis; F.vesca; G.hederacea; R.fructicosus (agg.); V.riviana; H.hummisifusum* and *L.campestris.* With the exception of *G.hederacea* these are the most frequent species seen throughout the woodland and are typical woodland species.

Table 5.38
Mean biomass per unit area by Ellenberg score for light (10^{-3} g cm^{-2}) +/- 1.s.e

Ellenberg Score	Treatment	Experiment 1	Experiment 2	Experiment 3
8	O_3 + Light	-	-	-
	CFA + Light	-	-	-
	O_3 + Shade	4.545 ± 1.1390	-	-
	CFA + Shade	4.298 ± 0.8050	-	0.32 ± 0.322
7	O_3 + Light	-	0.26 ± 0.013	0.40 ± 0.168
	CFA + Light	-	1.74 ± 0.077	0.53 ± 0.199
	O_3 + Shade	3.416 ± 1.0413	0.28 ± 0.113	0.48 ± 0.280
	CFA + Shade	2.741 ± 0.8534	0.28 ± 0.013	1.17 ± 0.467
6	O_3 + Light	-	0.77 ± 0.047	1.33 ± 0.917
	CFA + Light	-	0.08 ± 0.083	0.85 ± 0.362
	O_3 + Shade	0.648 ± 0.2607	0.43 ± 0.153	1.41 ± 0.777
	CFA + Shade	3.375 ± 1.2380	1.58 ± 0.533	0.27 ± 0.159
5	O_3 + Light	-	0.23 ± 0.157	0.11 ± 0.114
	CFA + Light	-	1.08 ± 0.049	0.05 ± 0.042
	O_3 + Shade	0.189 ± 0.0494	0.06 ± 0.063	0.02 ± 0.002
	CFA + Shade	0.877 ± 0.5578	0.03 ± 0.026	0.02 ± 0.013
3 & 4	O_3 + Light	-	0.26 ± 0.179	0.11 ± 0.106
	CFA + Light	-	1.08 ± 0.528	-
	O_3 + Shade	0.021 ± 0.0143	0.69 ± 0.652	3.00 ± 0.289
	CFA + Shade	0.003 ± 0.0016	0.28 ± 0.116	-

Experiment 1, stands out from the other experiments in sheer quantity and cover of biomass (Table 5.37 & 5.38). Biomass, cover, and number of species were much greater in Experiment 1 than either of Experiments 2 and 3. Experiment 1 had the largest difference in species number between treatments with many more represented in the O_3 treatment.

The division of final biomass between the Ellenberg classes varied between experiments in relation to both cover and biomass values of the species; the significance of these effects of ozone are summarised in Table 5.33. The balance of biomass between the Ellenberg groups showed large differences between the three experiments, which undoubtedly influenced the impacts of ozone.

In Experiment 1, biomass was dominated by species in Ellenberg groups 7 and 8; these are invasive, non-woodland species, the presence of which would decrease the conservation value of the woodland. Shade tolerant species (groups 3 & 4) were marginal in this experiment and were likely out-competed in this by group 7 & 8 species.

C.hirstuta was the only species present in the study belonging to group 8; in Experiment 1 this species alone contributed 58% in the O_3 shade and 38% CFA + shade, toward the mean final biomass. The results show a clear shift between the other classes due to ozone; with a decrease in biomass of Ellenberg groups 5 and 6 in ozone; whereas there was a non-significant increase in biomass (and to a greater extent cover) in groups 7 and 8. This implies that ozone caused a shift towards a community dominated by species that are less characteristic of woodland habitats.

The much greater productivity from Experiment 1 than the other experiments is potentially related to the shape of the pots used; these pots had greater exposure of the soil to the surface thus leading to a larger quantity of seed germinating. This may also

explain the larger proportion of open-habitat (groups 7-8) species in this experiment compared to the others, as these species are often stored for a long time in the soil as seed and disturbance of the seed bank will often stimulate germination. In comparison the pots in Experiment 2 & 3 were deeper and although germination may have occurred it may have been deep inside the pot and thus unsuccessful.

Experiment 2 shows a reduction in mean biomass per unit area for the ozone treatments. Experiment 2 was largely comprised of, and sourced from, a community of shade-tolerant woodland perennials, as would be expected; there was a greater contribution from Ellenberg classes 3 and 4, whereas the proportion of biomass in Ellenberg groups 7 and 8 was much reduced. Hence, the potential for more ozone-tolerant species to benefit from the reduced competitive vigour of more the sensitive species would be reduced. Indeed, ozone treatment reduced total biomass and cover and was associated with a reduced species richness, diversity index and equitability. This is similarly represented in the mean cover values (Table 5.37); from these figures there seem to be a clear treatment effect of ozone on this community which is independent of the effect of light. This is consistent with the results as very few measured parameters showed light treatment effects.

Ozone caused a decrease in biomass in all groups (except group 5 which was only a very small contribution) and there was no overall shift between Ellenberg groups. It should be noted that; (a) total above-ground production in Experiment 1 was four times greater in the control treatment than in Experiment 2; and (b) that ozone had a

significant overall effect on above-ground biomass in Experiment 2 but not in Experiment 1.

The results of Experiment 2, highlights the sensitivity of the characteristic woodland species to elevated ozone when not in a mixed community with other species. This suggests that they may be some effect of canopy cover and leaf area index on protecting these species within the other mesocosms experiments during ozone exposure.

Experiment 3 comprised a community not dissimilar to experiment 1, but with final biomass and cover values comparable with experiment 2. Table 5.38 shows that three out of four treatments were largely dominated by *R.fructicosus*; the exception being the CFA + shade treatment, which became dominated with rapid growing weed species namely *C.hirstuta* and *H.lanatus*. There is obviously a tendency within this community (Experiment 1 & 2) to be come dominated by light species, possibly due to the frequency of these propagules within the soil seed-bank. Differences in *R.fructicosus* frequency between mesocosms was not a result of treatment but an artifact of variable soil reserves. *R.fructicosus* so dominates the cover in the ozone treatments in Experiment 2 that all other species are marginal.

Where *R.fructicosus* was not present species diversity and equitability were slightly higher at the end of the experiment. There was a greater mean biomass per unit area of *R.fructicosus* in the ozone treatments in Experiment 3 and large cover values for

group 6 of which *R.fructicosus* was the largest contributor. Barbo *et al.* (1998) reported significant increases in cover of brambles (*R.cuneifolius*) during a two-season fumigation study on an early successional community such as in this study; furthermore, they reported that cover of brambles resulted in reduced species diversity and evenness (Barbo *et al.*, 1998).

As shown in Table 5.33 and 5.35, total biomass and cover were only significantly reduced by ozone in Experiment 2. This was in contrast to the results for the mesocosms from more open habitats, with a greater species number, for which there was no effect of ozone over time on species number and total cover, or on total biomass and biodiversity index. In these experiments, the impacts of ozone seemed to be greater on the relative prevalence of different groups of species. However, a significant adverse effect on biomass-based equitability was found in Experiment 1, reflecting a tendency to be dominated by one vigorous species, in most cases this was *C.hirstuta*. Dominance of the community by one or two vigorous species was definitely a characteristic of the mesocosms in Experiments 1 & 3.

5.4.3 Visible Injury

Table 5.39 gives a list of all species present in all three experiments and also gives details of observed foliar injury. The species are listed in order of their rank for the Ellenberg Index for light. Many species exhibited signs of stress in the ozone treatments and some showed specific ozone injury symptoms. Many species showed accelerated senescence and chlorosis, or developed red colouration. Three species, G.*hederacea*, *H.humifusum* and *Rubus* spp, showed typical flecking and stipple in all

three experiments, while *Schrophularia nodosa* and *Veronica chameadrys* showed such injury in the one experiment in which they were found.

In the experiments with individual plants of *G.hederacea* and *S.nodosa* (cf. Chapter 2), these species exhibited the same injury symptoms at a greater degree of severity. The other listed species showed injury in one experiment but not in others. Species within Group 6 showed some of the more severe symptoms of foliar injury. Within Group 6 was *G.hederacea;* this species was the most damaged in terms of visible foliar injury. In general there was no link between biomass reductions and visible injury.

Table 5.37:
Summary of species present in one or more mesocosm experiment:
'**a**' indicates species present exhibiting no foliar injury;
'**b**' indicates species present but exhibiting ozone specific injury;
'**c**' indicates species present but showing symptoms of stress.

Ellenberg Rank	Species	1	2	3
3	*Fagus sylvatica*		**a**	
	Mercuralis perrennis	**c**	**c**	**c**
	Urtica doica	**a**		
4	*Hyacinthoides non-scripta*	**a**	**a**	
	Oxalis acetosella	**c**		
	Veronica montana	**a**		**a**
5	*Aegopodium podagraria*		**c**	
	Geum urbanum		**c**	**c**
	Luzula pillosa	**a**		**c**
	Lysimachia nummularia	**b**		**a**
	Potentilla sterelis	**c**	**b**	**c**
	Primula vulgaris		**a**	
	Scrophularia nodosa	**b**		
	Silene doica	**a**		**a**
6	*Chamerion agustifolium*	**a**		**a**
	Deschampsia caespitosa	**c**		**a**
	Epilobium hirstutum	**a**		
	Epilobium montanum	**a**	**a**	
	Fragaria vesca	**c**	**c**	**a**
	Glechoma hederacea	**b**	**b**	**b**
	Holcus mollis	**a**		
	Ranunculus repens	**c**		**b**
	Rubus fructicosus agg.	**b**	**b**	**b**
	Valeriania officinalis	**c**		**a**
	Veronica chamaerodys	**b**		
	Viola rivinania	**c**	**c**	**c**

Table 5.37 cont.

Ellenberg Rank	Species	1	2	3
7	*Agrostis tenuis*	**c**		**a**
	Alchemilla glabra	**c**		**a**
	Cerastium fontanum	**a**		**a**
	Fillipendula spp.	**c**		
	Holcus lanatus	**b**		**a**
	Hypericum humifusum	**b**	**b**	**b**
	Juncus spp.	**a**		**a**
	Luzula campestris	**c**	**c**	**a**
	Plantago lanceolata	**b**		**a**
	Poa annua	**c**		**a**
	Potentilla erecta			**c**
	Prunella vulgaris	**c**		**a**
	Rumex acetosella	**c**		
	Rumex obtusifolium	**c**		**a**
	Sagina procumbens	**a**		**a**
	Stellaria media	**c**		**a**
	Trifolium repens	**c**		
8	*Cardamine hirstuta*	**c**		**a**

5.4.4 Individual Species responses

Characteristic woodland species (Groups 3 and 4)

Experiment 2 showed the only significant ozone associated reduction in biomass and cover of Ellenberg groups 3 & 4, for which they were a significant component of the community. In fact, these species were the largest contributors to biomass in CFA treatments in Experiment 2 (Table 5.36). The species, within this group, were particularly successful in CFA + light treatment (Table 5.38). In the O_3 + light treatment however, they are significantly reduced perhaps out competed by more vigorous species in group 6 and 7. In Experiment 3, these species are only present in

the ozone treatments and again the control treatments favour the light-loving species even in the shade.

The only species in these two groups exhibiting foliar injury was *M.perennis*, which is one of the more dominant species in woodland flora, showed non-specific injury in all of the experiments.

Open woodland species (Classes 5 and 6)

Dominance of these Ellenberg groups throughout all of the experiment was common (Table 5.36); furthermore they comprise nearly 50% of the top 5 biomass contributing species to all experiments. There were significant decreases associated with ozone treatment in the final live biomass of group 6 in Experiment 1, and in percentage cover of group 6 in experiment 2. The effects on group 6 were consistent with relatively high levels of ozone injury in this group. However there was quite variable species composition – only two species were found in all three experiments.

In experiment 2 and 3 group 6 generally had the greater biomass and cover values. Reductions in biomass between groups 6 and '3 and 4' seem to be in balance with each other in both ozone and control treatments. Suggesting these groups are in direct competition with each other. In the light, ozone favours group 6 and in the shade, favours groups 3 & 4. In experiment 3 in the ozone treatments group 6 seems to be the dominating group, outcompeteing all others as previously mentioned.

In Experiment 1, *Holcus mollis*, a group 6 species and a common species in woodlands and rides, was almost entirely lost from the ozone treatment. This species is shade tolerant and can persevere in a shady canopy. This species was present in both treatments but soon died off in the ozone treatment. In the control it survived and had large cover values; furthermore, it was one of the top five most dominant species in this experiment (Table 5.36).

Schophularia nodosa and *Glechoma hederacea*, are also common woodland flora species, both showed substantial visible injury in this experiment. Although these effects of ozone could not be tested significantly because of the lack of replication, it suggests that some individual species within this group could be at a competitive disadvantage under ozone stress.

There is a tendency for negative effects on group 5 in Experiment 1, but the groups contributed only a small component in the other experiments. Group 5 plays a very small role in experiments 2 and 3 with only very small cover and biomass values

Invasive, non-woodland species (Classes 7 and 8)

There were negative effects of ozone in Experiment 2 and 3 on this group but not in Experiment 1. Precedence of *C.hirstuta* in Experiment 1 clearly confounds effects; *C.hirstuta* was practically absent from experiments 2 and 3.

In Experiment 1 the ozone treatment favoured those species of open habitats, ranked under the Ellenberg group 7 and 8. At the end of the experiment, fewer species from group 7 were represented in the control treatment (10) than in the ozone treatment (15). The ozone treatment had a larger share of group 7 species in terms of higher species richness and abundance from the start of the experiment. This could be a chance distribution of propagules but could relate to changes in soil chemistry, composition, or biology caused by ozone and affecting germination potential. This had not been investigated and is merely speculative. In ozone, these species were also able to grow and become successful due to competitor release from species of mainly groups 5 and 6.

Species of group 7 seemed to show little response to ozone; only *P.lanceolata, Hypericum humifusum* and *H.lanatus,* showed visible injury, and there was little difference in biomass between the two treatments. However, in experiment 1 group 8 dominated most mesocosms. This group was actually just one species *C.hirstuta*; an open-habitat species, and was equally successful in terms of biomass, in both treatments. *C.hirstuta* was practically absent from experiments 2 and 3.

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Effects in Experiment 2 are surprising as group 7 species were not a major component; only two species were present, *L.campestris* and *H. humifusum*. *H. humifusum* had a tendency to become dominant in both shade and light conditions; However in the CFA + shade treatment in experiment 2, biomass of *H.humifusum* was much reduced and more shade tolerant species with a lower Ellenberg light rank were

more dominant (Table 5.36) and biomass of group 7 was much reduced (Table 5.38). It is probable that both species present are sensitive to ozone exposure, both species showed high degrees of ozone specific foliar injury; However, foliar injury on *H.humifusum* has not reduced its ability to quickly dominate vegetation especially under light conditions where it has a competitive advantage and there are not significant effects of biomass on this group.

However it seems that the lack of success of these species in Experiment 2 in the CFA + shade treatment is due to the ability of shade-tolerants (Ellenberg groups 3-6) to competitively exclude them; which they seem unable to do under light conditions and with the effects of ozone.

5.4.5 Modification by shade

Experiment 1

In Experiment 1, although there was no 'CFA + Light' and 'O3 + Light' treatments, the effect of ozone appear to be altered by the increased levels of shade. In the control treatment there was an increase in cover of groups 3 to 6, following the second shade. In the ozone treatment the second reduction in light cause a decrease in cover of all groups but then a steady increase in cover. Diversity calculated from cover was impacted by reduced shade but these related more to differences between individual mesocosms and their species rather than a treatment as whole.

5.4.6.2 Experiment 2

Experiment 2 had varied effects of shade; there were reductions over time in species richness, abundance, diversity and equitability. Foliar injury in ozone treatments was less in the shade in the early stages of Experiment 2, but increased in the later stages of the experiment to become similar to the light treatment. This suggests that in these early stages the species were under more stress in the light treatment.

In CFA, shade compared to light favours Ellenberg groups 3 and 4, over group 6 species, with the effect increasing over time. In contrast, with ozone present, the benefit of shade for groups 3 and 4 is largely lost, and there is only a small switch to groups 3 and 4 compared with 6 in ozone; hence, ozone interferes with the expected increased competitiveness of groups 3 and 4 compared to 6 with greater shade.

This effect is significant over time for Groups 3 and 4, for which shade limits increases in percentage cover over time (Fig 5.18). Many of the species in group 3 and 4 are known as 'shade-resistant' strategists and can tolerate shade due to the ability to persist in a vegetative state and slowing reproduction. These species typical of woodland would usually benefit from high light availability; during a post-coppice/ post disturbance event the perennial herbaceous flora has increased growth rates of those species able to react positively to light (Barkham, 1992). In the light control treatment there is a growth spurt seen in these species which is limited by the shade in those treatments and by ozone in the 'O_3 + Light' treatment.

5.4.6.3 Experiment 3

The shade treatments in Experiment 3 overall performed better than the mesocosms in the light. This was mainly due to the large abundance of group 7 and 8 species. These species grew vigorously and out-competed other species. The reason for these species preference to shade is a difficult to explain.

Experiment 3 had no significant differences in biomass of Ellenberg groupings except for group 7. Growth of species in group 7 was limited by the presence of ozone, leading to reductions in the biomass of these species in the ozone treatments. The shade treatments were higher in biomass of group 7 species the likely cause of this is just the nature of these fast growing weedy species reacting to the initial period of light and out competing members of other groups at the early establishment phase. Indeed in the 'CFA + Shade' treatment the presence of large amounts of group 7 & 8 species relates directly to reductions in other typical woodland species. In the ozone treatments the more typical woodland species seem to perform better, this could well relate to the sensitivity of the open habitat species to ozone and thus these groups have gained the competitive advantage.

5.4.7 Conclusions

Ozone has caused shifts in species composition, abundance and diversity in the experimental mesocosms. Ozone has led to symptomatic foliar injury appearance on plants within the ozone treated mesocosms. Some of these effects have been altered by different light environments. The direction of the interaction of the effects of ozone

and shade on woodland flora is difficult to assess, as there were variable responses between experiments. However, generally shade caused decreases in growth and diversity.

Much of the variation within and between experiments relates to stochastic responses of a plant 'in situ'. This variation was increased by allowing plants to germinate and develop as part of the experiment, but this small-scale heterogeneity is also a key feature of woodland habitats. The variable results in terms of response when comparing species, Ellenberg groups and experiments make it difficult to draw definite conclusions in terms of the likely impacts of ozone on woodland habitats. However, the experiments do demonstrate that ozone has significant effects on providing competitive advantages for certain species and opportunistic individuals. The results strongly suggest that the nature of the effect of ozone depends on the ecological context and species composition, and the nature and degree of competition.

6. Chapter 6: Discussion and Ecological Implications.

6.1 Introduction

This study sought to identify areas of sensitivity to ozone exposure in habitats of conservational importance in YDNP. The primary target was to identify those species and communities which may be at risk due to sensitivity to ozone exposure. In *Chapter 2 & 3*, woodland species in particular, and to a lesser extent grassland species, were identified as species in the YDNP sensitive to the effects of elevated ozone exposure. Risk, in terms of current exposure levels and stomatal conductance, was assessed at woodland and grassland sites in *Chapter 4*. Further experimental investigation took place to discover the impact of elevated ozone exposure on woodland ground flora in *Chapter 5* via ozone exposure of woodland ground flora mesocosms. The goal of the mesocosms was to create a simulated woodland community that was representative of the spatial heterogeneity that exists in this type of habitat. *Chapter 5* has given further evidence of sensitivity to ozone of woodland species under competitive interactions that exist in natural communities.

Much of the research described in this study focussed on woodland ground flora. For these reasons, this final discussion first identifies key findings of relevance to ozone impacts on semi-natural vegetation in general, and then those for relevance to woodland vegetation in particular. The findings for woodlands are then considered in a wider context, taking account of the impacts of climate change and woodland management. Finally, priorities for future research to better understand the impacts of ozone on upland semi-natural habitats and woodlands are identified.

6.1.1 Changes in ozone concentrations within plant canopies

Chapter 4 describes measurements of levels of ozone within the woodland canopy. Although a detailed study of ozone concentrations within a Norway spruce plantation were reported by Karlsson *et al.* (2006), there are no previous studies which have assessed ozone exposure at ground level in broadleaved woodland. Ozone levels within the woodland were reduced in comparison to outside of the woodland but were still relatively significant. However the low values of stomatal conductance recorded in typical species of the woodland ground flora suggest that ozone flux to the leaves will be small.

6.1.2 Between habitat differences in ozone sensitivity

There were systematic differences between habitats in their sensitivity to ozone; species (and in some cases, ecotypes) collected from drier, more open habitats, such as limestone pavements, were more resistant to ozone. It is possible that selection for survival in this type of habitat may confer some additional resistance to ozone for such species.

One way that plants adapt to dry environments is to reduce water loss by producing smaller leaves (Givnish, 1979) which reduce the transpiring leaf surface area. An additional potential adaptation is to change the relative rates of gas exchange to maximize the carbon assimilation to water-loss ratio, defined as the water-use efficiency (Cohen, 1970; Cowan, 1986). These hypotheses have been supported by comparative studies (Gurevitch *et al.* 1986; Kalisz and Teeri 1986; Ehleringer and Cooper 1988). The cumulative dose of ozone taken up by leaves, which is determined

by both stomatal conductance (g_s) and ozone concentration at leaf level, is assumed to be a key factor influencing ozone damage to plants (Pleijel *et al.*, 2004). Species with high values of stomatal conductance, such as wetland species, show sensitivity to ozone (Franzaring *et al.*, 2002; Power & Ashmore, 2002). It is likely that the reverse is true of drought resistant species and populations which show lower values of stomatal conductance. There is also evidence that drought tolerant Mediterranean species, particularly evergreen species, have a high tolerance to ozone which is due both to low rates of gas exchange and a high antioxidant capacity (Vitale *et al.*, 2007; Paoletti, 2006; Monk & Murray, 1995; Bussotti & Gerosa, 2002; Grulke & Paoletti, 2005; Nali *et al.*, 2004).

However, adaptation to dry, exposed conditions may not be a feature of the species living in the grikes and crevices in less exposed microclimates within the community, and thus these species may be more sensitive to ozone. In Chapter 2, the number of species studied from the limestone pavement habitat was small due to the difficult propagation of some species and the scope of this study. This meant that the study discounted many of the shade-adapted species of this habitat type, in particular, bryophyte species.

Bryophytes are an important component of limestone pavement and woodland communities, due to the shady and moist environment created in the pavement grikes. There is little research on the effects of ozone exposure on bryophytes. However, two ferns with wide occurrence in Eastern North America, *Athyrium felix-femina* and

Onoclea sensibilis; showed effects on spore germination when exposed to elevated concentrations of ozone (Bosley *et al.*, 1998). There is also evidence that ozone can reduce the growth rate of British bryophyte moss species (Potter *et al.*, 1996). Thus, it is possible that the species included within the study in Chapter 2 may not be representative of all components of the limestone pavement communities, and there may be more sensitive species in this type of habitat.

It is possible, that the lack of sensitivity shown by plants from Scar Close and Colt Park Wood (*c.f.* Chapter 2) could be due to resistance derived from past ozone exposure. In particular, *Lotus corniculatus* showed varied levels of sensitivity between the two woodland sites but resistance in the two limestone pavement populations from Ingleborough. Thus differences between sensitivity of species from different geographical regions of the Yorkshire Dales National Park (YDNP) may relate both to local adaptations to climatic conditions and to past ozone climate.

The higher sensitivity to ozone of many of the species from woodland systems in Chapter 2, 3 and 5 could thus derive from lower ozone exposure, or insufficient exposure to drive selection of resistance. Reduced concentrations of ozone will reach forest floor vegetation, reducing the impact of large seasonal peaks. However, this is likely only to be the case in the summer months when the canopy is closed. The winter-spring period is likely to be a time when ozone concentrations inside and outside the woodland are more similar, and this is the period that is critical for woodland herbs as it is the most active period of growth. This makes it less likely that

lower ozone exposures at sites such as Grass Wood are associated with higher ozone sensitivity. In addition, primarily light availability and canopy structure (Thomsen *et al.*, 2005) are likely to be the major ecological pressures driving selection in such a community.

The variation in ozone sensitivity between species found in the woodland mesocosm experiments could partly be explained by life history. For example, the low sensitivity to ozone of some of the more ruderal and short-lived perennial species such as *C.hirstuta*, may relate to the higher migration rate, shorter generation time and larger populations size of these species. In contrast, slow-growing stress-tolerant species typically expanding vegetatively will have very little introduction of new genetic material, low sexual reproduction and low migration rates, and thus may have little opportunity to develop ozone resistance.

6.1.3 Effects of Ozone on Root Biomass

An often neglected area of study is the effects of ozone on the root systems of semi-natural vegetation. A particular characteristic of upland vegetation is the tendency of these species to be long-lived perennials, with an ability to survive and increase in numbers using reproductive methods other than seed production (Jones, 2001).

This study identified several species which showed significant changes in below ground partitioning; to roots (cf. Chapter 2); 58% of species tested showed reductions in root biomass of 10% or greater with respect to the control. Furthermore, in spring flowering bulb species (Chapter 3), *A.ursinium* showed significant below ground

reduction in both bulb and root and the potential for a similar effect in *H. non-scripta* is evident. This study considered only short-term effects of ozone; the long-term consequence of adverse below-ground effects of ozone for populations of long-lived perennial species has not been considered in the literature and needs further investigation.

6.1.4 Changes in leaf morphology

Leaf morphology was altered by ozone through changes in plant form, leaf area, leaf width, and the number of leaves; these effects differed between species and populations. Changes in leaf shape may relate to the phenotypic plasticity of a species to adapt to environmental conditions.

Glechoma hederacea (c.f. Chapter 2) had a significantly reduced leaf area and many smaller leaves in ozone than in the control. The high degree of visible injury (specifically yellow specking over large areas of leaf surfaces) in this species, which can be attributed to cell death (Gravano *et al.*, 2003), would negatively affect its water balance. Increasing water use efficiency, by reducing leaf area and leaf size, would reduce the overall area for transpiration, as in species with drought tolerance. Thus a plastic response which might have evolved as a potential adaptation to drought dress could also provide protection or resistance to ozone.

Plantago lanceolata plants showed reductions in the width of the leaf in ozone in two populations. This could potentially be an adaptation to reducing the available leaf area for transpiration. In addition an overall switch to an 'upright' phenotype in both

populations was observed in the ozone treatment. Different phenotypes of *P.lanceolata* relate to the type of community and management of the grassland; upright forms are more common in meadow communities whereas prostrate forms are more typical of grazed grasslands. These adaptations would respectively increase competitiveness for light and space in meadows and reduce the extent of grazing. Why the change to greater frequency of the upright form occurs under ozone exposure in *P.lanceolata* is unclear. However if such switches occur due to ozone exposure in a natural community setting, they may have implications for 'fitness' to the environment by altering the plants' competitive ability. 'Upright plants' in a grazed meadow, for example, will be at a higher risk of grazing damage.

6.1.5 Effects of ozone on species number and diversity

Effect of ozone exposure on individual plant species have been extensively studied, but little is known of the effects of exposure on communities and especially effects on woodland ground flora communities. The findings from Chapter 5 show that ozone can cause changes in species number and diversity within such communities. Few studies have demonstrated this effect for ozone; the only comprehensive study on the effects of ozone exposure on ground flora by Barbo *et al.* (1998) showed that species diversity and evenness were reduced in an ozone exposed early successional woodland community. Similarly to the findings of Ashmore and Evans (1992), changes in species cover were largely related to changes in cover of the dominant species; in the study by Barbo *et al.* (1998) the dominant species was bramble (*R.cuniefolius*). This tendency for ozone exposed communities to quickly become dominated by vigorous species was also a property of the experiments in *Chapter 5*.

6.2 Ozone effects on woodlands and woodland ground flora.

6.2.1 Community Composition

Changes in community composition have been noted in several previous studies on community responses to ozone (Bassin *et al.*, 2006; Ramo *et al.*, 2006; Samuelsson *et al.*, 2006; Ashmore & Ainsworth, 1995; Barbo *et al.*, 1998; Ashmore & Evans, 1992). In Chapter 5, changes in abundance, cover and species number over time were all altered by ozone treatment. Identifying which species were consistently affected was difficult, as species composition varied between individual mesocosms, and competition between individual neighbouring plants is likely to alter plant response to ozone exposure. Thus predicting which individual species will be impacted most by elevated exposure in a woodland community is difficult from this study.

Hayes *et al* (2006b) conducted a meta-analysis of the ozone sensitivity of 83 species of semi-natural vegetation. They suggested that species with a high Ellenberg light score (6+) were more sensitive to ozone, although this was based on above-ground biomass only, and included both positive and negative effects. In Chapter 2, the majority of the species studied were within the Ellenberg Light groups of 5-8; 57% of the species tested showed some response to ozone exposure, although the most important was reduction in below-ground biomass. However, in Chapter 5, in a competitive setting there was very little negative impact of ozone exposure on the light adapted species (Ellenberg classes 6-8); there is therefore little evidence in these experiments to suggest that light-adapted species are any more affected by ozone

exposure than the shade species. It is clear that the influence of competition alters the effects of ozone for some species.

The lack of effects on light-adapted species in Chapter 5, compared with Chapter 2, could be due to the fact that these species are partitioning resources to the above ground parts of the plants over investment to later reproductive output or to root biomass; there could therefore be losses in biomass below ground which were not measured in the experiments of Chapter 5. A second possible factor is the influence of competition. It is difficult to relate other ozone studies to these experiments as very few species present in the mesocosms have been tested for sensitivity to ozone or in a competitive scenario. In general, there was a greater tendency for species of shade and semi-shade to be out-competed by more ruderal species when exposed to ozone. The changes in species composition in Chapter 5 seem to derive, at least in part, from competitive interactions and thus identifying particular ecological groups' sensitivity to exposure is difficult due to the variability of the competitive interactions in the individual mesocosms.

Overall, from the data in Chapter 5, is seems that the species typical of a woodland community, namely Ellenberg groups 3-6, are at a disadvantage when exposed to elevated levels of ozone. Under these conditions there are reductions in biomass and cover and unknown effects on root biomass and seed production. These species tend to possess some shade tolerance, but are not strong competitors and thus may be

unable to withstand consecutive seasons of high ozone exposure. They may thus become quickly excluded from their natural habitat by strong competitors.

6.2.2 Ecological implications of below ground reductions in biomass

In Chapters 2 and 3, changes in biomass below ground were a significant feature of many of the species exposed to ozone. For annual species, the reduction of root biomass may not have severe consequences as there is no requirement for a long-term viable root system; many annual species will flower quicker if conditions are unfavourable e.g. during a drought, and a shorter lifespan is generally part of these species' adaptations. However for the perennial species which comprise the majority of woodland herb species, the implications may be more serious.

Vernal spring flowering species such as *H. non-scripta* are adapted to woodland habitats through a temporal shift in photosynthetic period; these species are most active when the tree canopy is dormant or beginning to leaf. For example, *H.non-scripta* prepares initials for leaves and flowers within the storage bulb by July and roots are regrown by September (Blackman and Rutter, 1964), so any reductions in assimilation or an unsuccessful growing season caused by high levels of ozone could potentially cause serious knock-on effects for the following season's growth.

Shade species tend to have very heavy seeds and dispersal is limited to a few meters or centimetres. For example, observed dispersal rates for *H.non-scripta* have been reported as ranging from 0.006 m y^{-1} to 0.06 m y^{-1} (Van Der Veken *et al.*, 2007); Rackham (1980) suggests colonization rates of c. 100 m per century, in contrast,

Pigott (1982) records figures of 6–10 m per century and Honnay *et al.* (1999) report an average colonization distance of 32 m, and a maximum of 55 m, per century.

Recovery of communities is likely to derive from the presence of perennials and dispersal from reservoirs. Loss of woodland perennial species is more permanent, and studies of the recovery of ground flora specifically have suggested that re-colonisation is unlikely to occur (Brown and Warr, 1992). A number of woodland plants, including many rare species, are virtually confined to ancient semi-natural woodland (Peterken, 1979) and do not quickly expand their range.

Changes in root structure and carbon partitioning to the roots caused by ozone may have knock-on effects on the soil and its microbial community. There have been many studies looking at changes in the mycorrhizal communities in response to ozone exposure; some of these studies have highlighted an increase in mycorrhizal fine roots, and increases in abundance and diversity of these communities (Grebnec & Kraigher, 2007a), which are related to a reduction in N uptake and nutrition (Haberer *et al*, 2007). However, some have reported reductions in root mycorrhiza and diversity (e.g. Zeleznik *et al.*, 2007). Many of these changes to the below ground microbial community have been attributed to changes, mainly reductions, in carbon allocation to the roots (Grebnec & Kraigher, 2007b), and thus less energy being transferred to the microbial communities. Indeed for many of the species mentioned in Chapter 2 there were significant reductions in biomass below ground; If ozone exposure results in reductions in root biomass this could be detrimental to community

survival, especially for some 'woodland indicator' species such as *H. non-scripta*, which are dependent on associated mycorrhiza for phosphorous uptake (Merryweather & Fitter, 1995).

6.3 Ozone and Climate Change

6.3.1 Climate Change and British woodland.

The present ranges of many of our British woodland communities are predicted to move northward in response to the changing climate (Broadmeadow *et al.*, 2005; Wesche *et al.*, 2006; Harrison *et al.*, 2001). This conflicts with conservation priorities which tend to favour maintaining the status-quo and actively discriminate against trees not typically located in the community.

Kirby *et al* (2005) found changes in species distributions and abundance over the last 30 years that were correlated with climate change; they suggest that as changes are already being observed in the phenology of species, it is likely that effects on woodland species abundance will become even more common in the next 50 years as the climate changes. As predictions for climate change stand for woodlands, canopy species will change as the south of England becomes drier and condition become unsuitable for native species; in particular the range of beech, *Fagus sylvaticus*, is predicted to move North and West (Wesche *et al.*, 2006).

Such changes in the dominant tree species may significantly affect the associated ground flora species; these may struggle to re-establish as many woodland species are

relatively poor colonisers (Peterken, 1974; Bossuyt *et al.*, 1999; Kirby *et al.*, 2005) and woodland cover in Britain is highly fragmented. It has been suggested that over the next 50 years any significant changes in rainfall patterns are likely to have a greater effect on the distribution of rare species of plants and animals in the UK than the predicted changes in temperature (Elmes and Free, 1994).

For beech woodland, Wesch *et al.* (2006) suggest that new communities of ground flora will form, different to those initially native to the region and from the initial range of the beech. They also suggest that climate change will affect the distribution of woodland ground flora independently of its effects on the tree canopy. The experiments in Chapter 5, which show significant effects of ozone in the initial phase of establishment of seed or perennial structures, imply that this may be a particularly sensitive phase and that ozone could in future be a constraint to the re-establishment of characteristic ground flora communities in new locations as the climate changes.

6.3.2 Ozone and elevated carbon dioxide

It is also been predicted that the direct effects of rising concentrations of carbon dioxide will enhance photosynthesis, and experimental evidence indicates a growth enhancement of UK species of 30 –50 per cent for young trees in response to a doubling of ambient CO_2 concentrations (Broadmeadow & Randle, 2002). However, timber production in the U.K. is predicted to be adversely affected by the effects of drought over-riding the beneficial effects of rising CO_2 levels, longer growing seasons and increased solar radiation inputs (Broadmeadow *et al.*, 2005).

Hence effects of ozone in woodlands need to be considered in the context of increased CO_2 concentrations. Information on the long-term effects of ozone and CO_2 in combination on a young plantation of poplar, birch and maple is provided by the Aspen FACE project, in the Northern USA. This field fumigation experiment used increased levels of CO_2, ozone and the combination of both, that were based on forecasted levels for the year 2050 in the USA (King *et al.*, 2005). Relative to the control, elevated CO_2 increased total biomass 25, 45 and 60% in the aspen, aspen–birch and aspen–maple communities, respectively, while O_3 caused 23, 13 and 14% reductions in total biomass relative to the control in the respective communities. Combined fumigation resulted in total biomass response of 7.8, +8.4 and +24.3% relative to the control in the aspen, aspen–birch and aspen–sugar maple communities, respectively. These results indicate that exposure to O_3 may significantly reduce the capacity of tree growth to respond positively to elevated CO_2 in some forests.

However, the effects on ground flora of this combination of O_3 and CO_2 have not been reported, and there is a clear need to consider how differential positive responses to increased CO_2 concentrations of the different groups of species considered in the experiments in Chapter 5 might modify the effects of ozone. Indirect effects may also occur through the effects of elevated CO_2 if increased growth and biomass of the canopy species decreases light and ozone penetration to the forest floor.

6.4 Management

Woodlands have been managed by man since as early as Neolithic settlements (Ingrouille, 1995). Coppice woodlands, such as Grass Wood, that have been coppiced since 1600, are considered as ancient semi-natural woodland (ASNW) and deemed historically important. Such woodlands are believed to be the relics of the natural climax woodland of Britain (Peterken, 1981), dating back to the Atlantic period (Ingrouille, 1995). The natural regenerative nature of many tree species when cut has led to the development of the coppice cycle. Evans (1992) defines coppicing as ' a coppice is a forest crop raised from shoots produced from the cut stump (called stools) of the previous crop, and coppicing is the operation of regenerating crops this way'.

The stand structure in coppice woodlands gives rise to the structural diversity and the habitat mosaic which is important in attracting the diverse range of plants and animals present in these communities (Warren & Fuller, 1990). The action of coppicing creates a cyclic change in the ground flora. This means that the woodland is periodically regenerated, allowing colonisation and succession to take place and offering a diverse habitat. ASNW contain disproportionately more of the rare, local and native species of plants and invertebrates (Peterkin, 1992). Conservationists state that regular rotational coppicing is the best method to maintain the high biodiversity and species of conservation importance in ASNW (Butcher, 1980) and there is emphasis of continuity of practice.

There are two distinct phases to the coppice cycle:

i) the high light phase (open canopy) and;

ii) the closed canopy phase.

Light increases two-fold in spring and increases twenty-fold in summer (Rackham, 1975) during the high light phase. Sudden changes in conditions after coppicing cause an influx of light demanding species; thus at the beginning of the coppice cycle the community is most diverse and this progressively decreases with increasing shade leaving only the shade-tolerant flora at the closed canopy phase. Dependent on the tree species within the coppice stands, it takes approximately 3-4 years for the canopy to close (Barkham, 1992). The vegetation of the coppice cycle is subjected to progressive alteration in structure through the cycle. Due to the repetition of the same sequence every time the canopy is cut, similar plant communities recur at intervals in the same place, and this pattern is called cyclic succession or secondary succession (Grime, 2002). The driver for successional change is primarily the disturbance created by coppicing and then increased shade.

There is no research specifically assessing the effect of ozone exposure in coppice woodlands. It is possible given the stress effect of coppicing to trees, and the reported effects of ozone on: *F.sylvatica* (*e.g.* Stribley and Ashmore, 1992) a common coppice species; that coppice stools may be sensitive to ozone.

In terms of woodland ground flora regeneration, Ash and Barkham (1976) suggest two phases in the field layer succession: after coppicing 'firstly, the establishment

from seed and vegetative propagules of a mixed community; secondly, the loss of species incapable of perenniation and in increase in those capable of vegetative reproduction'. Shade tolerant perennials and vernal species such as *H.non-scripta* and *Anenome nemorsa*, respond quickly with increased flowering and seed production during the high light phase (Brown and Oosterhuis, 1981) and increase in above ground biomass (Ford & Newbold, 1977).

Evidence from the experiments in Chapter 5 suggests, firstly, that the perennial species (essentially the shade tolerants) may be out-competed by invading light species during the high light phase, while under closed canopy coppice conditions; there may be direct adverse effects of ozone on the shade tolerant species. Hence, the results from the experiments carried-out in this project suggest the potential for adverse effects of ozone in both phases of the coppice cycle.

However, this assessment is only based on effects observed over a single generation. Light species largely comprising of species buried in the seed-bank from the previous rotation (Brown and Ooserhuis, 1979) are unlikely to be affected by carry-over effects of previous ozone exposure. However, studies of the germination potential of seeds produced from ozone exposed plants (*e.g.* Bender *et al.*, 2006), have demonstrated the potential for ozone to cause reduced seed output and reduced germination ability of seeds from ozone exposed parent plants.

In addition, many of the perennial species already *in situ*, and the species migrating from transient populations in woodland rides and glades, may be affected by past ozone exposure. It is therefore possible that these species are the most likely to decrease in population size under elevated ozone, from both past effects of exposure and from direct effects on growth under current exposure. The coppice cycle has preserved these species for many hundreds of years, creating a light phase in which to reproduce and then eliminating competition due to the increasing shade; however the benefit to these species of the light phase will largely be lost under conditions of elevated ozone if they become increasingly dominated by vigorous herbs and annual species.

6.5 Conservation

Continuity of woodland management is beneficial to the conservation of associated plants and animals; cessation of management typically results in densely shaded tree canopies and loss of biodiversity (Brown and Warr, 1992; Brown and Oosterhuis, 1981). Grass Wood is mixed upland woodland comprising of mixed stands of hazel coppice (*Corylus avellana*) and mature or regenerating oak (*Quercus spp*,), beech (*Fagus sylvatica*), sycamore (*Acer psuedoplatinus*) and ash (*Fraxinus excelsior*). Although the structure of the woodland has been extensively modified by replanting, the site maintains a rich ground flora, for which the site is principally valued (Natural England, 2007). Among the woodland flora species associated with limestone outcrops are several locally uncommon species including rock whitebeam (*Sorbus rupicola),* and angular Solomons Seal (*Polygonatum odoratum).*

Management directions for UK upland woodland stands under recommendations from Natural England (2007) suggest re-introduction of coppicing in woodland where there are direct benefits to biodiversity, otherwise management as a high forest system. Coppicing in Grass Wood has been re-introduced over the past 15 years, and Natural England now report the status of the site as 99% favourable recovering, suggesting that the site is recovering from the last 100 years of neglect.

The implications of rising ozone levels may suggest that woodland communities may become quickly dominated by vigorous species such as brambles and open-habitat ruderals. The greatest effect of ozone on ground-flora, post-coppicing when conditions are light, is likely to be during the most sensitive time for woodland perennials. In terms of management this may involve some weeding out of vigorous species, and clearing rides periodically, to reduce competition to perennials, and to sustain a wider diversity of micro-habitats; this will help reduce the impacts of ozone for sensitive species. Introduction of low impact (and low density) grazers such as cattle or pigs may help management especially in areas of high forest, as these species typically create gaps in vegetation. Animals play an important role in semi-natural vegetation and they have long associations with plants for pollination and seed dispersal; in terms of management they play a vital role in creating heterogeneity in a habitat.

A study by Honnay *et al.* (2006), on the effect of habitat fragmentation on genetic diversity of *A.vulneria*, provides indirect evidence that management by grazing not

only positively affects habitat quality but that it might also mitigate the genetic consequences of habitat fragmentation. This study highlights the importance of grazing, and of the regular transport of livestock between fragments, to prevent the long-term effects of fragmentation on the genetic diversity of populations (Honnay *et al.*, 2006).

6.6 Priorities for Future Research

This is the first study to consider in any detail the potential impact of ozone on woodland ground flora communities of Atlantic Europe in general, and on upland deciduous woodlands in particular. It has only been possible to carry-out a limited amount of work focussed on one particular region and wood. Therefore the results can only be described as preliminary, and wider extrapolation to effects of ozone on woodland ground flora in general can only be made with considerable caution.

Nevertheless, the results do suggest that the effects of ozone on woodland ground flora may be significant and the rising background levels of ozone that are predicted for remote upland areas of the UK may pose a significant future threat to these communities. As identified by this research and by Barbo *et al*, (1996) ozone may cause changes in the species composition of the ground flora community in ways that are difficult to predict at present, and may depend on site-specific conditions. Hence, further research is needed, and some key priorities are considered below.

1. A more integrated approach to assessment of effects on trees and on woody and herbaceous ground flora is needed. Changes in ozone exposure can have significant effects on individual tree species, leading, amongst other things, to significant: foliar injury, changes in the growth patterns of the canopy, reduced growth and reductions in carbon partitioning to the roots. All these effects in a woodland community are likely to have knock-on effects for the ground flora. For instance, a change in inputs to the soil from decreased carbon assimilation and translocation by the trees is likely to have an impact on the soil microbial community and chemistry, with implications for nutrient availability, while later bud emergence and earlier leaf fall may increase light levels at the woodland floor in the early and late growing seasons.

2. Shade exerts a major control on species composition of woodland ground flora and more research is needed to assess how this may modify the ecological impacts of ozone. The work described in Chapter 5 has shown trends for effects of ozone on competitive interactions and species richness to be altered by levels of shade. Furthermore, the community from the coppice area showed quite different responses from those from the clearing and ride areas. Kirby *et al.* (2006) describe how UK woodlands are becoming shadier habitats due to lack of management. The implications of this alone for the rich diverse flora of woodlands is severe; a high forest stand will have low species diversity naturally in the U.K. comprising of the shade-tolerant flora only e.g. *H. non-scripta, M. perrenis*. Increasing effects of ambient ozone levels have the potential to further exclude flora from the woodland floor. An interacting factor is the extent to which the density and structure of the

woodland canopy modifies the ozone exposure of ground flora. The results in Chapter 4 suggest that ozone levels inside the woodland at ground flora level can be similar to those outside. Research is therefore needed to further assess how canopy structure and levels of shade may modify both responses of woodland species to ozone and the concentrations of ozone to which they are exposed.

3. *Many of the species of the woodland flora are sensitive to ozone exposure and it seems that the characteristic 'woodland indicator' species such as the spring flowering bulbs may be particularly sensitive.* There is therefore an urgent need for longer term experiments under more natural conditions, to assess the sensitivity of key woodland indicator species to ozone; such work has recently been initiated at the University of Newcastle. This should be extended to other woodland perennial species and those classed as 'woodland indicator' species with low dispersal ability, for example Herb paris (*Paris quadrifolia)*, Angular solomons seal (*Polygonatum odoratum*), Lily of the valley (*Convallaria majalis*).

4. *More research is needed to assess the subtle changes in plant form which may offer resistance to ozone and through which ozone may change the fitness of a population.* This study revealed morphological adaptations to leaves, and phenotypic variants present in plant populations, which seem to offer some protection to ozone exposure; conversely in one case, ozone changed morphological form in a way that would be beneficial in some habitats but detrimental in others. Identification of

resistant populations of wild species will help conservationists with the many challenges ahead with a changing climate.

5. *More research is needed into the effect of ozone on bryophyte species common to these habitats to obtain a comprehensive assessment of ozone effects on woodland ecosystems.*

Abbreviations

ANOVA	Analysis of Variance
BAP	Biodiversity Action Plan
BW	Bastow Wood
CFA	Charcoal filtered air
COP	Coniston Old Pasture
C-S-R types	Competitive-Stress Tolerant-Ruderal types (see Grime, 2001)
IRGA	Infrared Gas Analyser
NEGTAP	National Expert Group on Transboundary Air Pollution
NFA	Non filtered air
NNR	National Nature Reserve
NVC	National Vegetation Classification
O_3	Ozone
PAR	Photosynthetically Active Radiation
ppb	Parts per billion
RAMSAR	Ramsar sites are wetlands of international importance designated under the Ramsar Convention.
RS	Relative Sensitivity
SPSS	Statistical Package for the Social Sciences
SSSI	Site of Special Scientific Interest
YDNP	Yorkshire Dales National Park

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