Research article

Ultraviolet radiation modulates C:N stoichiometry and biomass allocation in Fagus sylvatica saplings cultivated under elevated CO_2 concentration[☆]

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ABSTRACT

Under the conditions of ongoing climate change, terrestrial ecosystems will be simultaneously exposed to a permanent rise in atmospheric CO_2 concentration and increasing variability of such environmental factors as temperature, precipitation, and UV radiation. This will result in numerous interactions. The interactive effects caused by exposure to such multiple environmental factors are not yet well understood. We tested the hypotheses that enhanced UV radiation reduces the stimulatory effect of elevated CO_2 concentration on plant biomass production and that it alters biomass allocation in broadleaved European beech (*Fagus sylvatica* L.) saplings. Our results after 2 years of exposure confirmed interactive effects of CO_2 concentration and UV radiation on biomass production, and particularly on biomass allocation to roots and aboveground biomass. The strongest stimulatory effect of elevated CO_2 on aboveground biomass and roots was found under ambient UV radiation, while both low and high UV doses reduced this stimulation. Nitrogen content in the roots and the distribution of nitrogen among leaves and roots were also significantly affected by interaction of CO_2 concentration and UV radiation. The observed changes in leaf and root C:N stoichiometry were associated with altered morphological traits, and particularly with a change in the proportion of fine roots. As the biomass allocation and especially the proportion of fine roots can play an important role in effective water and nutrient use and acclimation to future climates, it is essential to obtain a deeper understanding of the links between C:N stoichiometry and biomass accumulation.

1. Introduction

As a result of global climate change, environmental conditions in terrestrial ecosystems are also changing. In particular, there are clear long-term trends of increasing CO_2 concentration ([CO_2]) and mean annual temperature (IPCC, 2013). Additionally, short-term, extreme fluctuations of environmental factors will become more common, and these include extreme precipitation distribution (drought periods vs. flash floods) and extreme temperatures (heat waves vs. late frosts) (Trnka et al., 2009). Furthermore, variations in ultraviolet (UV) radiation due to seasonal changes in concentrations of stratospheric ozone, aerosols, and/or cloudiness are also expected (Gandia et al., 2015). Terrestrial ecosystems will thus be simultaneously exposed to a multitude of environmental signals and/or stressors (Mittler, 2006). Whilst there is a good knowledge base concerning the impacts of

individual environmental factors on plants, the interactive effects caused by exposure to multiple factors are not yet well known. Consequently, the prediction of ecosystem responses and acclimation under climate change is usually subject to significant error. To minimize uncertainties in the predictions, a new type of multifactorial impact studies focusing on interactive effects under a wide range of interacting factors is required (Ballaré et al., 2011; Way et al., 2015).

The Representative Concentration Pathways (RCPs) predict atmospheric CO_2 concentrations to reach between 421 μmol mol^{-1} (RCP2.6) and 936 μmol mol^{-1} (RCP8.5) by the end of the 21st century (IPCC, 2013). Over the past two decades, extensive data have been generated on plant responses to elevated [CO_2] (reviewed in Urban, 2003; Leakey et al., 2009; Way et al., 2015). These data show that even in the absence of other environmental changes increases in [CO_2] induce rather complex, species-specific responses in plants. These include both

positive and negative effects depending on plant ontogeny, duration of the $[CO_2]$ treatment, and/or nutrient and water availability. Given the complexity of $[CO_2]$ responses, it can be expected that interactions with other factors may trigger an even broader range of responses. Furthermore, while some of these interactions will synergize plant $[CO_2]$ responses, others may be antagonistic (Ainsworth and Rogers, 2007; Larsen et al., 2011). Ultraviolet and particularly UV-B ($\lambda = 280$–315 nm) radiation can substantially modify the effect of elevated $[CO_2]$ on plants and terrestrial ecosystems (Ballaré et al., 2011). In plants, UV radiation induces a variety of responses, including DNA modifications and photorepair, formation of reactive oxygen species, accumulation of photoprotective compounds and antioxidants, and morphological changes (Lavola et al., 2000; Robson et al., 2015). Given the need to predict the ability of plants to acclimate to realistic, future climate conditions, it is necessary to understand the interactive effects of elevated $[CO_2]$ with other environmental drivers, and particularly with light spectral composition and intensity of UV radiation.

It is generally hypothesized that high UV doses reduce the positive effects of $[CO_2]$ on photosynthesis and biomass production (Poorter and Pérez-Soba, 2001). On the other hand, several studies have shown compensating effect of elevated $[CO_2]$ on UV-B damage (Koti et al., 2007). In general, the interactive effects involving $[CO_2]$ and UV radiation vary greatly according to species, genotype, and UV dose (reviewed by Caldwell et al., 2007). While significant ameliorating effect of elevated $[CO_2]$ on photosynthetic parameters has been found under intermediate UV-B doses, it completely failed to ameliorate negative effects of high UV-B doses (Zhao et al., 2003). The UV dose is thus assumed essential in determining the final direction of interaction with $[CO_2]$. Such conclusions can be also inferred from a comparison of plant responses to elevated $[CO_2]$ measured in different exposure systems. It has been argued that the relatively strong $[CO_2]$ responses measured in laboratory growth chambers and/or field open-top chamber experiments are associated with a lack and/or substantial reduction of UV-exposure (de Graaff et al., 2006). Conversely, substantially lower rates of $[CO_2]$-enhanced photosynthesis and plant growth have been measured in plants grown in Free-Air $[CO_2]$ Enrichment (FACE) systems (Nowak et al., 2004; Körner et al., 2005; Leakey et al., 2009) where the plants are exposed to natural UV doses.

These hypotheses on the interactions between $[CO_2]$ and UV are backed up by a small amount of experimental evidence. Experiments in which plants were exposed to different combinations of $[CO_2]$ and UV irradiance revealed that photosynthetic quantum efficiency in rice, soybean, and wheat is stimulated by elevated $[CO_2]$ only at low UV irradiances (Teramura et al., 1990). On the other hand, $[CO_2]$ enrichment may induce the protection of photosynthetic reactions against UV radiation via increased leaf thickness (Stewart and Hoddinott, 1993), reduced oxygenase activity of Rubisco enzyme (Šprtová et al., 1999), and/or increased accumulation of secondary metabolites linked to defence and repair (Ainsworth and Rogers, 2007; Ballaré et al., 2011). Antagonistic interactions of $[CO_2]$ and UV, and particularly UV-B, radiation on total plant biomass have been reported (Sullivan and Teramura, 1994; Rozema et al., 1997). These effects are species-specific (Teramura et al., 1990; Urban et al., 2006) or dose-specific (Zhao et al., 2004), however, and may differ with treatment duration (Teramura et al., 1990; Lavola et al., 2000).

Plant $[CO_2]$ responses, and in particular $[CO_2]$ acclimation, are highly dependent on the plant nutrient status (McMurtrie et al., 2008; Leakey et al., 2009). It is therefore critical to understand how interactive effects of $[CO_2]$ and UV affect root development. To date, only a few studies have investigated the impacts of combined $[CO_2]$ and UV on root growth, particularly in tree species (Bussell et al., 2012; Robson et al., 2015). Moreover, these earlier studies must be interpreted with caution, as they often used rather high UV doses and ecologically unrealistic ratios between UV-B, UV-A, and photosynthetically active radiation. For example, Sullivan and Teramura (1994) reported a significant interactive effect of UV-B and $[CO_2]$ on biomass allocation in

loblolly pine. The root-to-shoot ratio was reduced by high UV-B doses at ambient $[CO_2]$ but increased at elevated $[CO_2]$. Ziska and Teramura (1992) found, however, an opposite interaction between UV-B and $[CO_2]$ in rice. While the root-to-shoot ratio increased at elevated UV-B radiation and ambient $[CO_2]$, it substantially decreased at elevated $[CO_2]$. Among the possible explanations for such discrepancies is that different UV doses were applied. Studying cotton plants, Zhao et al. (2004) showed that changes in root and total plant biomass depend on UV dose. While root and total plant biomass increased at ambient UV irradiances in comparison to a UV-excluded treatment, both parameters displayed significant decreases at double ambient UV intensity irrespective of $[CO_2]$.

Plant morphology and biomass allocation are related to the C:N stoichiometry of plants. The stoichiometry of C:N is also associated with the relative abundance of secondary metabolites (Poorter and Nagel, 2000; Sulpice et al., 2013), and accordingly to a development of plant defence mechanisms against photo-oxidative stress (Lavola et al., 2000; Klem et al., 2015). Both UV radiation and $[CO_2]$ affect carbon (C) and nitrogen (N) balance in plants (Leakey et al., 2009; Sardans et al., 2012), and therefore it can be assumed that interactive effects between $[CO_2]$ and UV radiation are going to affect the C:N ratio. Thus, C:N stoichiometry seems to be one of the integrating factors between UV radiation and $[CO_2]$ effects on biomass accumulation and allocation. Indeed, substantially reduced enhancements of photosynthesis and growth by high $[CO_2]$ have been observed after long-time exposure (Urban, 2003; Leakey et al., 2009; Way et al., 2015). Such decline is often attributed to a limited N availability (Körner et al., 2005; McMurtrie et al., 2008). Accordingly, a detailed understanding of the role of C:N stoichiometry in interactive effects of environmental factors is critical for understanding the effects of elevated $[CO_2]$ on global plant growth.

In this study, we tested the hypothesis that enhanced UV radiation reduces the stimulatory effect of elevated $[CO_2]$ on plant biomass production. We further hypothesized that UV radiation alters biomass allocation, thus leading to changes in root-to-shoot ratios. The hypotheses were tested on saplings of broadleaved European beech (*Fagus sylvatica* L.) cultivated for two consecutive growing seasons under two $[CO_2]$ and three UV radiation intensities. It was expected that such long-term treatment would lead to an altered C:N ratio in aboveground and belowground tissues, as well as altered sink:source balance in plants.

2. Material and methods

2.1. Plants and experimental design

The experiment was carried out at the Bílý Kříž experimental research site in the Beskydy Mountains, Czech Republic (49°30′N, 18°32′E, 908 m a.s.l.). The area has a cool (annual mean air temperature 6.8 °C) and humid (annual mean relative humidity 84%) climate with high annual precipitation (average for 1998–2011 was 1293 mm). Microclimatic parameters were measured during the 2015 and 2016 growing seasons and are shown in Table 1.

European beech (*Fagus sylvatica* L.) is a broadleaved tree species that is widespread in the temperate zone, including the Beskydy Mountains. Saplings (3 years old and approximately 0.4 m high at the beginning of the experiment) were grown within experimental lamellar domes (Šigut et al., 2015). The lamellas are made from UVT Solar acrylic material (Quinn Plastics, Enniskillen, UK) transmitting more than 90% of incident UV-A and UV-B radiation. The saplings were grown in a native soil (Mesozoic Godula sandstone – flysch type) at ambient (400 μmol CO_2 mol^{-1}; hereafter AC) or elevated (700 μmol CO_2 mol^{-1}; hereafter EC) atmospheric $[CO_2]$ for 2 years. To achieve stable EC conditions, $[CO_2]$ inside the dome was monitored by an LI-840A infrared gas analyser (Li-Cor Biosciences, Lincoln, NE, USA). The determined $[CO_2]$ was subsequently used for feedback regulation of a mixing module.

Table 1

Monthly sums of photosynthetically active radiation (ΣPAR), ultraviolet-A radiation (ΣUV-A), and ultraviolet-B radiation (ΣUV-B); and daily mean air relative humidity (RHa), air temperature (Ta), and soil temperature (Ts) for individual months of the 2015 and 2016 growing seasons inside glass domes. Measurements were made automatically at 10 min frequency.

		Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
2015								
ΣPAR	MJ m^{-2}	175.84	187.93	241.78	273.52	241.51	128.63	81.51
ΣUV-A	MJ m^{-2}	12.02	12.88	16.84	18.72	16.12	8.62	5.13
ΣUV-B	MJ m^{-2}	0.71	0.81	1.11	1.28	1.08	0.53	0.27
RHa	%	76.1	82.7	78.0	67.2	63.6	83.8	90.5
Ta	°C	5.7	10.4	14.8	18.9	20.2	12.0	7.0
Ts	°C	4.4	10.6	15.0	17.7	18.3	14.2	9.3
2016								
ΣPAR	MJ m^{-2}	157.77	221.00	253.12	228.83	217.36	167.81	61.45
ΣUV-A	MJ m^{-2}	8.91	12.27	14.79	13.94	13.13	9.48	3.68
ΣUV-B	MJ m^{-2}	0.64	1.02	1.25	1.16	1.04	0.74	0.28
RHa	%	80.8	75.8	75.0	80.9	83.3	81.1	96.9
Ta	°C	6.6	12.0	16.4	16.7	15.1	14.7	5.1
Ts	°C	6.7	10.5	15.5	17.3	16.9	15.2	8.6
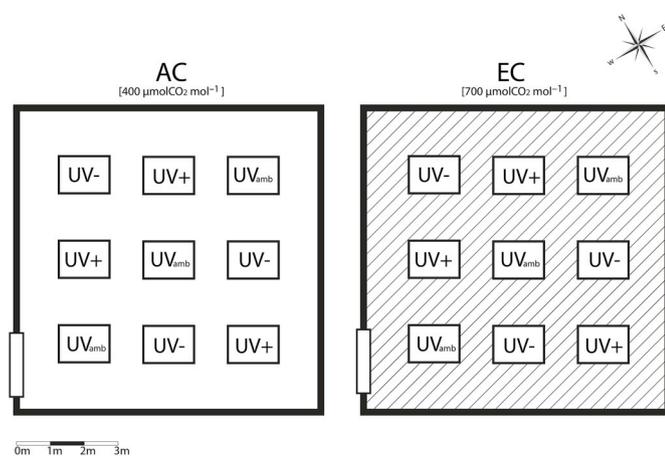
Fig. 1. Experimental design scheme. AC, ambient CO_2 concentration; EC, elevated CO_2 concentration; UV–, exclusion of UV-B and UV-A radiation; UVamb, ambient (non-manipulated) UV intensity; UV+, UV-B and UV-A radiation enhanced by 150% as compared to UVamb treatment.

Technical details are explained in Urban et al. (2001) and Šigut et al. (2015). CO_2 enrichment under the dome was continuous from April to November each year. Both AC and EC domes were split into three blocks (replications) within which the UV treatment plots were randomized (see the experimental design in Fig. 1). Three plots representing UV exclusion [UV–], three plots representing ambient UV [UVamb], and three plots representing enhanced (150% of ambient) UV radiation [UV+] were thus established.

Above each experimental plot (1.0 × 0.75 m) a lamp-bank (approximately 1.20 m height) was built and it was covered by a plastic filter. [UV–] plots were covered with clear plastic Lee U.V. 226 filters (Lee Filters, UK) to exclude UV-A and UV-B radiation, while [UVamb] and [UV+] plots were covered with diacetate foil. The filters covered the top and upper one-third of the lamp-bank walls to prevent penetration of direct solar radiation and to avoid cross-contamination between individual UV treatments. A modulated UV lamp system (Konel, Zlín, CZ) was used to achieve enhanced UV intensities (Klem et al., 2015). The system consists of three UV-A (TL 20 W/10 SLV; Philips) and four UV-B (TL 20 W/12 RS SLV; Philips) fluorescent lamps per plot. Incident UV-A and UV-B irradiance was monitored under the lamp-bank using SKU 420 and SKU 430 sensors (Skye Instruments, Powys, UK). The outputs of both UV-A and UV-B lamps were adjusted to a specified dose using a feedback and amplification circuit. To avoid

irradiation with UV-C radiation (< 280 nm), the UV fluorescent lamps were wrapped in pre-solarized (8 h) cellulose diacetate film 0.13 mm thick.

Daily biologically effective UV-B doses (UV-B$_{BE}$) were close to zero under [UV–] treatments, while UV-B$_{BE}$ was in the range of 11.6–20.5 kJ m^{-2} day^{-1} under [UV+] treatment, depending on actual sky conditions and on the season. UV-B$_{BE}$ was calculated from the emission spectrum of the UV lamps in the range 200–980 nm as measured by a SM 9000 spectroradiometer (PSI, Brno, CZ) and from an action spectrum for plant growth inhibition (Flint and Caldwell, 2003). Monthly sums of ambient photosynthetically active radiation (ΣPAR), UV-A (ΣUV-A), and UV-B (ΣUV-B) radiation during the growing season are shown in Table 1. LI-190 quantum sensors (Li-Cor Biosciences), located at the top of the canopies, were used to determine the actual intensity of incident PAR. Generally, the 2015 growing season was warmer and with higher intensities of PAR and UV radiation, particularly in July and August, compared to 2016. During the cooler 2016 growing season, the warmest months were May and June.

2.2. Plant harvest

Saplings were carefully harvested at the end of the 2016 growing season. The complete root ball was collected. Nine saplings from each experimental plot were used for further morphological analyses. The length of the aboveground part, diameter at the stem base and diameter at 5 cm above the base were measured immediately after harvest under the field conditions. Under laboratory conditions, total aboveground biomass, woody aboveground biomass, leaf biomass (LB), and root biomass (RB) of investigated saplings were determined. The remaining soil was washed away from the roots. The roots were separated into different fractions according to root diameter: ≤ 1 mm, 1–2 mm, 2–5 mm, and 5–20 mm. There were no roots with diameter > 20 mm. All parts were dried at 80 °C to a constant weight, then weighed. Roots of diameter ≤1 mm plus roots of diameter 1–2 mm were defined as fine roots (FR).

Fresh leaves were scanned before drying to estimate the projected leaf area. The scans were analysed using the program ImageJ (Schneider et al., 2012). Leaf mass per area was calculated as the ratio of leaf dry weight to leaf area. Furthermore, the following ratios were calculated: FR to total root biomass, FR to total leaf biomass, and root to shoot biomass.

Four to five leaves and roots from 4 to 5 saplings per plot were sampled, dried to a constant weight in a drying oven (80 °C) for approximately 2 days, then homogenized in an MM200 ball mill (Retsch, Haan, Germany). The analyses of C and N content in leaf and root samples (ca 100 mg) were performed using an automatic elemental analyser Flash 2000 (Thermo Scientific, Waltham, MA, USA).

2.3. Statistical analysis

Dixon's Q test was used for identifying and rejecting outliers ($p = 0.05$). Factorial multivariate analysis of variance (MANOVA) was used for the general analysis of [CO_2] and UV effect on biomass production, biomass allocation, C:N stoichiometry, and their mutual interactions. Wilks' lambda was used for multivariate statistical significance testing. F-ratio was then used for univariate effect size testing. Tukey's HSD post-hoc test ($p = 0.05$) was used to analyse any significant differences between treatments. The statistical analyses were conducted using Statistica 12 software (StatSoft, Tulsa, CA, USA). Bar graphs with confidence intervals and the multiple scatter graphs with regression lines were created in the software SigmaPlot 11.0 (Systat Software, San Jose, CA, USA). The biplot of redundancy analysis (RDA) was set up in the software CANOCO 5 (Microcomputer Power, Ithaca, NY, USA) in accordance with Šmilauer and Lepš (2014).

Table 2
Summary of univariate significance test (*F*-ratio, MANOVA) of the influence of CO_2 concentration ([CO_2]), UV radiation, and their combination ([CO_2] × UV) on European beech biomass and C:N stoichiometry. Statistically significant effects and interactions are indicated in bold ($p \leq 0.05$).

	[CO_2]	UV	[CO_2] × UV
Leaf biomass [g DW]	**< 0.001**	0.511	0.217
Leaf area [m²]	**< 0.001**	0.365	0.343
Woody aboveground biomass [g DW]	**< 0.001**	0.587	0.110
Total aboveground biomass [g DW]	**< 0.001**	0.450	0.123
Basal stem diameter [mm]	**< 0.001**	0.327	0.058
Plant height [cm]	**< 0.001**	0.467	0.170
Roots ≤ 1 mm [g DW]	**0.022**	0.259	0.120
Roots ≤ 2 mm [g DW]	**< 0.001**	0.191	0.285
Roots > 2 mm [g DW]	**< 0.001**	0.744	0.858
Root biomass [g DW]	**< 0.001**	0.787	0.529
Roots ≤ 2 mm/Root biomass	0.547	**0.033**	**0.019**
Roots ≤ 2 mm/Leaf biomass	0.695	**< 0.001**	0.173
Root/Shoot	0.793	0.066	0.482
Leaf C [%]	0.066	0.363	0.566
Root C [%]	0.897	0.354	0.621
Leaf N [%]	**0.001**	**< 0.001**	0.276
Root N [%]	**0.004**	**0.007**	**0.016**
Leaf C:N	**< 0.001**	**< 0.001**	0.412
Root C:N	**0.013**	**0.045**	0.052
Root C/leaf C	0.489	0.304	0.745
Root N/leaf N	**< 0.001**	0.077	**0.005**
Root C:N/leaf C:N	**0.001**	0.247	**0.023**
Total root N/total leaf N	**0.013**	0.429	0.117

3. Results

The factorial multivariate analysis of variance (Wilks' lambda, MANOVA) revealed statistically significant effects of both [CO_2] and UV radiation and also significant interactive effects generally on biomass formation, biomass allocation, and C:N stoichiometry ($p < 0.001$ for [CO_2] effect and [CO_2] × UV radiation interaction; $p = 0.0016$ for UV radiation effect). On the other hand, univariate significance testing (*F*-ratio, MANOVA) revealed for biomass production parameters statistically significant effects only for [CO_2], while the effects of UV radiation were not statistically significant (Table 2).

Following two growing seasons and irrespective of UV exposure, EC saplings had greater total aboveground biomass (Fig. 2) as well as belowground biomass (Fig. 3) than did AC saplings. The total root biomass was as much as 39.2% greater under EC than AC conditions. EC saplings had as much as 44.8% greater total aboveground biomass as compared to their AC counterparts. EC saplings were also taller and had greater

basal stem diameter, although the variation of these traits was rather large.

3.1. Aboveground biomass

All aboveground morphological parameters studied were significantly ($p \leq 0.005$) affected by [CO_2] (Table 2). In contrast, UV radiation had no significant effect on aboveground biomass. Although no statistically significant interactive effects of [CO_2] and UV radiation were observed, the effect of [CO_2] on most aboveground biomass parameters varied with UV radiation intensity. In general, the differences between AC and EC were greatest under UVamb but reduced under UV+ and UV– conditions (Fig. 2). For example, the leaf area per tree was distinctly larger under EC as compared to control (AC) conditions, but the stimulatory effect of EC was greatest under UVamb while under UV– and UV + it diminished (63.1% increase in [EC UV–], 72.9% in [EC UVamb], and 50.2% in [EC UV +] plants) (Fig. 2B).
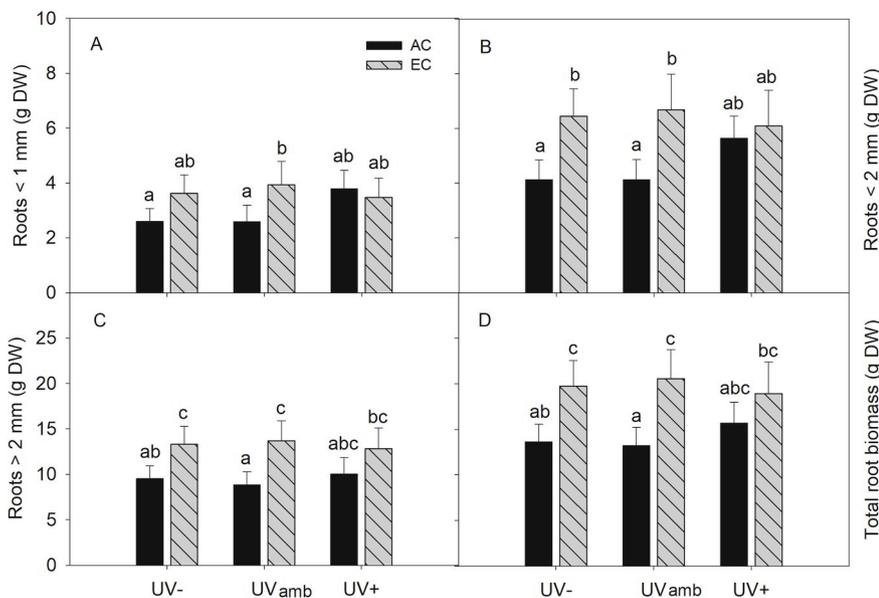
3.2. Belowground biomass

Belowground biomass accumulation was significantly influenced by [CO_2] (Table 2), with greatest total root biomass produced by EC plants. The greatest amount of root biomass was achieved in the [EC UVamb] treatment. The percentage increase in root biomass varied by root fraction. Under [EC UVamb], the increase in biomass of roots with diameter ≤ 1 mm was by 51.9%, that for ≤ 2 mm was by 62.0%, and for > 2 mm it was by 54.6% compared with the control ([AC UVamb]). When comparing the [AC UV +] treatment with ambient UV conditions [AC UVamb], it is evident that FR under enhanced UV radiation were influenced more than were the roots with diameter > 2 mm (RB of roots with diameter ≤ 1 mm was greater by 46.3%, that for ≤ 2 mm was greater by 36.9%, and for > 2 mm it was by 113.5% compared to [AC UVamb]). Apart from the effects of [CO_2] and UV on FR, a significant interactive effect of [CO_2] and UV on FR (diameter ≤ 1 mm) development was also observed.

It is clear that under UV– and UVamb conditions there was significantly greater root growth in EC than in AC (Fig. 3). Enhanced UV radiation (UV +), however, reduced this stimulatory [CO_2] effect. Roots with diameter > 2 mm responded similarly as did woody aboveground biomass (Figs. 2C and 3C). It can be summarized that the effect of [CO_2] and UV dose was mostly reflected by the growth of FR with diameter ≤ 1 mm (Fig. 3A) and ≤ 2 mm (Fig. 3B).
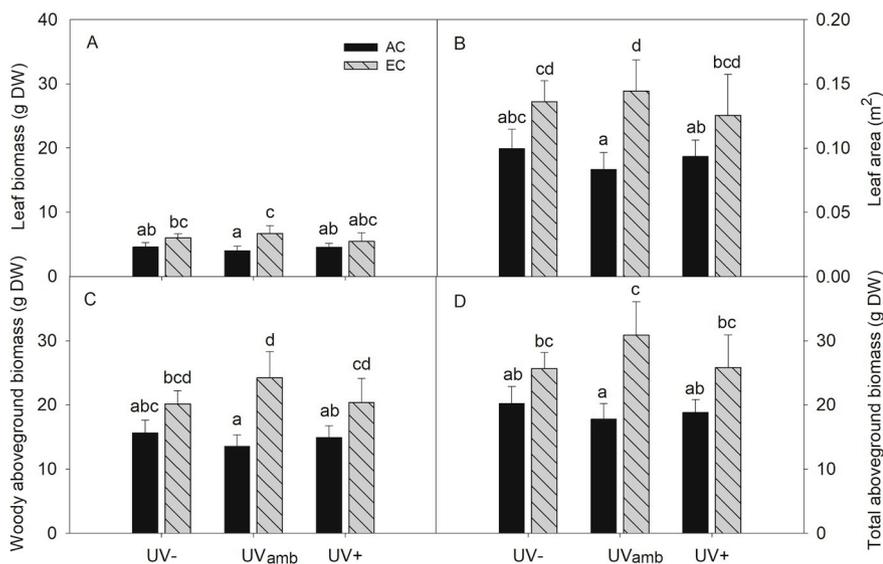
Fig. 2. Changes in leaf biomass per tree (**A**), leaf area per tree (**B**), woody aboveground biomass (**C**), and total aboveground biomass (g DW) (**D**) of *Fagus sylvatica* saplings treated under ambient (AC) and elevated (EC) CO_2 concentrations and different UV radiation doses. UV– represents exclusion of UV-B and UV-A radiation, UVamb represents ambient UV intensity, and UV + represents UV-B and UV-A radiation enhanced by 150% as compared to UVamb treatment. Columns are mean values, vertical bars represent 95% confidence intervals, $n = 3$ (experimental plots). Different letters indicate significant differences ($p \leq 0.05$) estimated on the base of Tukey's ANOVA post-hoc test.

Fig. 3. Changes in belowground biomass of *Fagus sylvatica* saplings treated under ambient (AC) and elevated (EC) CO_2 concentrations and different UV radiation doses. Biomass of fine roots of diameter $\leq 1\,mm$ (**A**), roots of diameter $\leq 2\,mm$ (**B**), roots of diameter $> 2\,mm$ (**C**), and total root biomass (**D**) are presented. UV– represents exclusion of UV-B and UV-A radiation, UVamb represents ambient UV intensity, and UV + represents UV-B and UV-A radiation enhanced by 150% as compared to UVamb treatment. Columns are mean values, vertical bars represent 95% confidence intervals, $n = 3$ (experimental plots). Different letters indicate significant differences ($p \leq 0.05$) estimated on the base of Tukey's ANOVA post-hoc test.

3.3. Biomass allocation

The ratios between FR and total RB, between FR and LB, and between total RB and total aboveground biomass were investigated (Fig. 4). The FR-to-RB ratio was significantly higher in [AC UV +] than in the corresponding control ([AC UVamb]) treatment (Fig. 4A). Values of the FR-to-LB ratio tended to increase ($p \leq 0.05$) with an increasing UV dose (Fig. 3B). This increase was more pronounced under AC than EC conditions. No effects of [CO_2] and UV radiation on root-to-shoot ratio were found (Fig. 4C).

3.4. Stoichiometry of root and leaf C and N

Nitrogen (N) content in both leaves and roots was significantly affected ($p \leq 0.01$) by [CO_2] and UV radiation. By contrast, no statistically significant effect of [CO_2] and UV radiation was found on leaf or root C content (Table 2, Fig. 5A and B). A significant interaction between the effects of [CO_2] and UV radiation ($p \leq 0.05$) on root N content was also found. Greater leaf N content was observed in AC compared to EC plants, whereas root N content was greater under EC compared to AC treatment. The only significant difference ($p \leq 0.05$) in leaf and root N content, however, was found when comparing EC with AC plants exposed to UV– conditions (Fig. 5C and D). Changes in N content thus played the key role in the C:N ratio in leaves and roots, because the C content was found to remain constant under all treatments. Accordingly, the only significant difference ($p \leq 0.05$) in C:N ratio between EC and AC plants was found under the UV– treatment (Fig. 5E). The interactions between effects of [CO_2] and UV radiation on the C:N ratio were not significant in leaves and were close to the significance threshold in roots ($p = 0.052$).

Fig. 6 shows C and N allocation between belowground and aboveground plant organs. A significant difference ($p \leq 0.05$) was detected in the ratios representing N allocation between roots and leaves (root N/leaf N, root C:N/leaf C:N and total root N/total leaf N; Fig. 6B, C and 6D respectively) when comparing EC and AC plants exposed to UV– and/or UVamb conditions. The ratio remained unchanged, however, under the UV + treatment. The UV + treatment thus tended to impede the reallocation of N towards the roots. Such a significant ($p = 0.005$; Table 2) interactive effect of [CO_2] and UV radiation indicates an antagonistic interaction of these two factors. Similarly, a significant ($p = 0.016$; Table 2) antagonistic interaction between [CO_2] and UV radiation treatments on changes in leaf N content was observed (Fig. 5D). Unlike [CO_2] and UV radiation effects on root N/leaf N, these factors had no effect on the ratio of C distribution between roots and leaves (root C/leaf C; Fig. 6A).

3.5. Relationships between C:N stoichiometry and morphological traits

Redundancy analysis (RDA) (Fig. 7) shows the associations between plant traits (morphological parameters and C:N stoichiometry) and treatments ([CO_2] and UV radiation). The main biomass production parameters total aboveground biomass, total root biomass, individual root fractions, leaf area, and leaf biomass were positively associated with elevated [CO_2]. Moreover, biochemical parameters like root N/leaf N and total nitrogen content per plant were positively linked with elevated [CO_2]. On the other hand, negative effects of [CO_2] were found for the C:N allocation between roots and leaves (root-to-leaf ratio) and C:N ratio in roots. The effects of UV radiation on morphological traits and C:N stoichiometry were less pronounced as compared to the [CO_2] effect. The RDA scores for leaf C:N ratio indicate similar effects of both [CO_2] and UV radiation.

Leaf C:N ratio exhibits a positive association with the main morphological parameters. This ratio is also influenced by UV treatment. Therefore, we tested the relationships between C:N ratio in both leaves and roots and corresponding total leaf and root biomass (Fig. 8). Although the relationship between leaf C:N ratio and total aboveground biomass was statistically significant ($p \leq 0.05$) only for the UV– treatment, it is obvious that these relationships are shifted with increasing UV dose to higher C:N ratios. In addition, under the UV– treatment the slope was less steep as compared to those for UVamb or UV +. There is a negative relationship between root C:N and total root biomass which is significant for the UVamb treatment. The shift caused by UV radiation is less evident for root biomass, and the slope is least steep for the UV– treatment.

RDA analysis (Fig. 7) indicates an interactive effect of [CO_2] and UV radiation on the root-to-leaf ratio of N content. Therefore, we analysed how N reallocation influences development of total leaf, aboveground, and root biomass under individual UV treatments (Fig. 9). These relationships are statistically significant in the case of the UVamb treatment (at $p \leq 0.05$ for total aboveground biomass and at $p \leq 0.01$ for total leaf and total root biomass) and also for that of the UV– treatment for total aboveground biomass and total leaf biomass ($p \leq 0.05$). The variability of the root-to-leaf ratio of N content is rather small under the UV + treatment and, as a result, the relationships with the three biomass parameters are not statistically significant. The slope of these relationships between UV and biomass steepens with increasing UV dose, and this is particularly evident for the UV– and UVamb treatments.
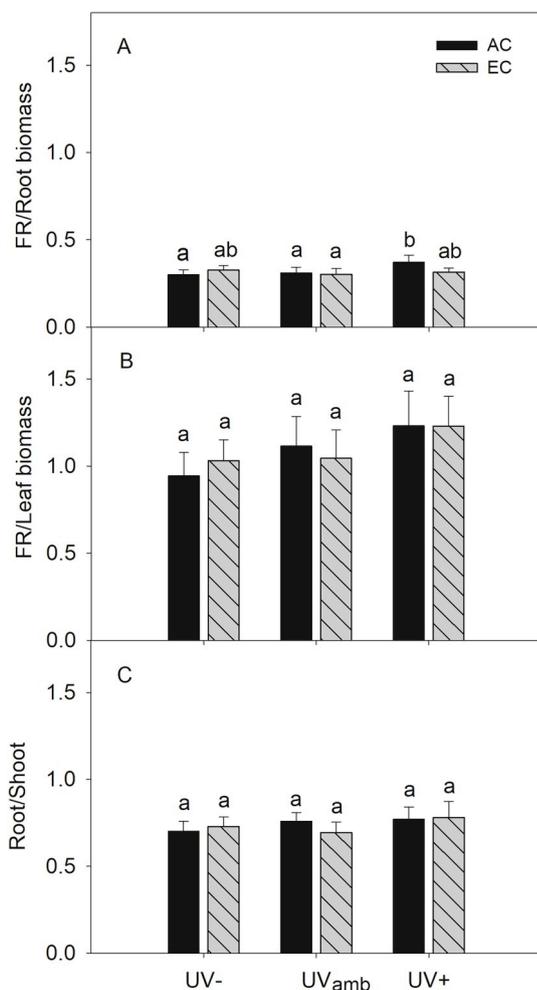
Fig. 4. Changes in biomass allocation in *Fagus sylvatica* saplings treated under ambient (AC) and elevated (EC) CO_2 concentrations and different UV radiation doses. The ratios of fine roots (FR) with diameter ≤ 2 mm to total root biomass (**A**), fine roots (FR) with diameter ≤ 2 mm to leaf biomass (**B**), and total root biomass to total aboveground biomass (Root/Shoot; C) are presented. UV– represents exclusion of UV-B and UV-A radiation, UVamb represents ambient UV intensity, and UV + represents UV-B and UV-A radiation enhanced by 150% as compared to UVamb treatment. Columns are mean values, vertical bars represent 95% confidence intervals, $n = 3$ (experimental plots). Different letters indicate significant differences ($p \leq 0.05$) estimated on the base of Tukey's ANOVA post-hoc test.

4. Discussion

4.1. Biomass production and allocation

Factorial multivariate analysis of variance (MANOVA) revealed significant general effects of both UV radiation and [CO_2] on biomass formation and allocation, as well as significant interactive effect of these two factors. Univariate analyses, however, show distinct effects of UV radiation and [CO_2] on individual traits (Table 2 and Figs. 2 and 3), with generally more pronounced effects of [CO_2] and significant effects of UV radiation and interactions only for root traits. High UV doses increased accumulation of root biomass, and particularly of FR under AC. Under EC, however, this stimulatory effect was not observed. UV radiation had only minor effects on aboveground biomass under both AC and EC conditions. The strongest response of aboveground biomass to EC was found for UVamb as compared to UV– and UV + treatments. The data thus allow us to assume a bell-shaped UV dose-response pattern for stimulatory effect of [CO_2]. The belowground biomass response to EC also was greatest under the UVamb treatment. The UV– treatment

induced only small changes in [CO_2] stimulation, however, and thus the bell shape of the UV dose-response curve is skewed to high UV doses. These data indicate that biomass allocation to above- and belowground biomass also is affected by interaction of UV radiation and [CO_2]. Poorter and Pérez-Soba (2001) had shown low negative interaction between UV radiation and [CO_2], which means that high UV-induced stress leads to low stimulatory effect of elevated [CO_2]. They also found great variability among individual studies, however. Our data confirm such negative effect of higher UV doses on [CO_2] response in general, but it also points to an essential role of UV dose, which, in addition to interactions with other environmental factors, can help to explain the great variability among studies. High UV doses (particularly of UV-B) reduce the [CO_2] stimulation mainly through negative effects on photosynthesis, either due to a direct impact on photosystem II or to reduced chlorophyll content (Kakani et al., 2003). Another reason for the negative impact of UV radiation on responses to [CO_2] can be assumed to be its inhibition of elongation growth via the UVR8 signalling pathway (Heijde and Ulm, 2012; Robson et al., 2015). On the other hand, EC generally increases contents of soluble phenolic compounds (Peñuelas and Estiarte, 1998; Jaafar et al., 2012) that play important roles as UV screening compounds and/or as antioxidants (Klem et al., 2012; Schreiner et al., 2012). Also, increased accumulation of primary metabolites under elevated [CO_2] can be used as energy sources for protective and reparative mechanisms under the conditions of oxidative stress (Ainsworth and Rogers, 2007) and thus may boost plants' tolerance to high UV doses. Such complex and multiple interactions are therefore likely the main reasons for different interactive effects of UV radiation and [CO_2] found in different studies varying in UV dose. In agreement with a meta-analysis carried out by Poorter and Nagel (2000), we found a weak effect of EC on biomass reallocation among roots, stems, and leaves. On the other hand, it is widely accepted that UV-B radiation has a positive effect on root-to-shoot ratio (reviewed by Robson et al., 2015). For example, Deckmyn et al. (1994) reported an increased root-to-shoot ratio in bean plants under conditions of enhanced UV-B, but a high intensity of photosynthetically active radiation significantly modulated this response. Although, similarly to Lavola et al. (2000), we found no significant effect of UV radiation on root-to-shoot ratio, the effect of UV on this trait was stronger as compared to that of [CO_2].

Although a positive effect of [CO_2] on the root-to-shoot ratio has been documented by a number of studies (reviewed by Rogers et al., 1999), it is obvious that interactive effects with UV radiation can greatly alter the effect of [CO_2] on this ratio (Bussell et al., 2012). Indeed, while in our study enhanced UV reduced this ratio under conditions of ambient [CO_2], the ratio increased under elevated [CO_2]. This interactive effect was not statistically significant, however. Our results show an effect of UV radiation on FR, and particularly roots ≤ 1 mm, thus indicating that most affected are actively growing roots. Based on a meta-analysis of 140 recent studies, Li et al. (2010) concluded that there is a stronger effect of UV radiation on root-to-shoot ratio in short-lived herbaceous species as compared to woody species. Such effects are likely related to UV-induced decrease in auxin metabolism and/or transport (reviewed by Jansen, 2002). Although these mechanisms are not yet fully understood, a lowered content of auxins results in reduced plant height and increased branching of the root system (Brown et al., 2001) and leads thus to UV-induced changes in plant morphology and biomass allocation (Hectors et al., 2012).

Antagonistic interactive effects of [CO_2] and UV radiation on morphological parameters are most commonly reported (reviewed by Ballaré et al., 2011). This means that enhanced UV radiation reduces stimulatory effects of elevated [CO_2]. Such a trend is evident also from our results. There are only a few studies indicating positive interaction (either additive or synergistic) of [CO_2] and UV radiation on plant morphology. For example, Teramura et al. (1990) reported a synergistic interaction of [CO_2] and UV radiation on aboveground biomass in soybean, but this interaction was antagonistic in wheat and rice. Based
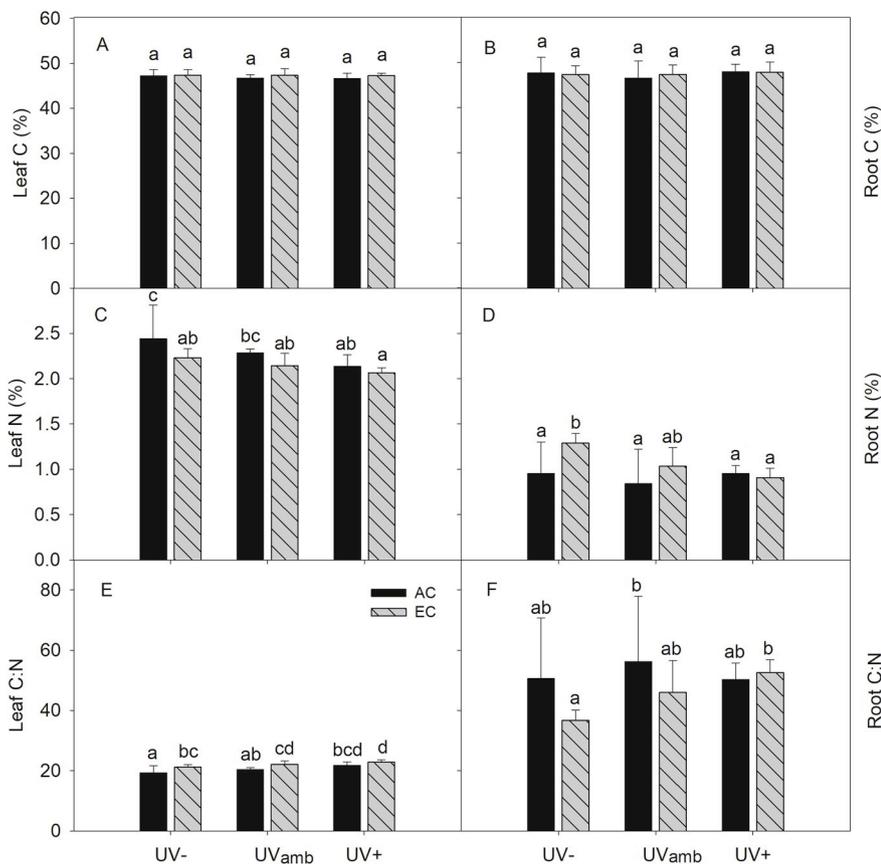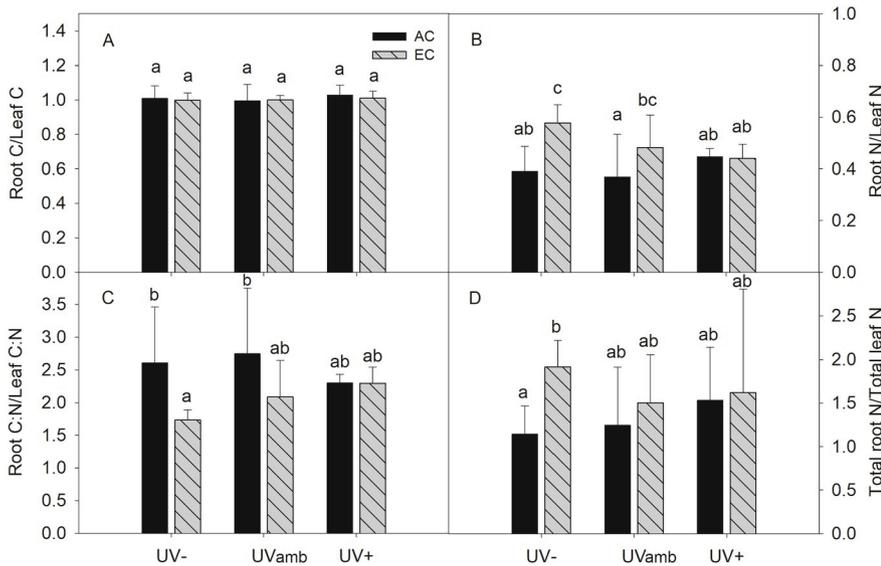
Fig. 5. Changes in carbon (C) and nitrogen (N) content per unit dry mass and C:N stoichiometry in leaves (**A**, **C**, **E**) and roots (**B**, **D**, **F**) of *Fagus sylvatica* saplings treated under ambient (AC) and elevated (EC) CO_2 concentrations and different UV radiation doses. UV– represents exclusion of UV-B and UV-A radiation, UVamb represents ambient UV intensity, and UV + represents UV-B and UV-A radiation enhanced by 150% as compared to UVamb treatment. Columns are mean values, vertical bars represent 95% confidence intervals, *n* = 3 (experimental plots). Different letters indicate significant differences ($p \leq 0.05$) estimated on the base of Tukey's ANOVA post-hoc test.

on the bell shape of the UV dose-response curve for EC stimulation, we can assume that interactions range from positive (additive or synergistic) under low UV dose to antagonistic under high UV dose.

Several physiological processes and metabolic pathways have been identified as contributing to such interactive effects of [CO_2] and UV radiation. These include in particular stomatal conductance and biosynthesis of primary and secondary metabolites, especially saccharides, amino acids, and flavonoids. While elevated [CO_2] generally reduces stomatal conductance (Urban, 2003; Ainsworth and Rogers, 2007), the effect of UV radiation seems to be dose- (Zhao et al., 2004) as well as species-specific (Teramura et al., 1990; Urban et al., 2006) and may be

further modulated by other environmental factors like light intensity, vapour pressure deficit, and wind velocity leading to both stomata opening (Kostina et al., 2001; Qaderi and Reid, 2005) and closure (Urban et al., 2006). In this experiment, light-saturated stomatal conductance of EC plants remained reduced as compared to that of AC plants across the entire vegetation season and for all UV treatments (Urban et al., this issue). Moreover, we found that while UV radiation stimulates a positive effect of EC at the beginning of the vegetation season, long-term cultivation under conditions of EC and enhanced UV leads to down-regulation of photosynthesis and reduced biosynthesis of carbohydrates.


Fig. 6. Changes in root-to-leaf ratios of carbon (**C**), and nitrogen (N) contents (**A** and **B**, respectively), C:N (**C**), and total N content (**D**) estimated in *Fagus sylvatica* saplings treated under ambient (AC) and elevated (EC) CO_2 concentrations and different UV radiation doses. Total N content is defined as total amount of N in all leaves or roots. UV– represents exclusion of UV-B and UV-A radiation, UVamb represents ambient UV intensity, and UV + represents UV-B and UV-A radiation enhanced by 150% as compared to UVamb treatment. Columns are mean values, vertical bars represent 95% confidence interval, *n* = 3 (experimental plots). Different letters indicate significant differences ($p \leq 0.05$) estimated on the base of Tukey's ANOVA post-hoc test.
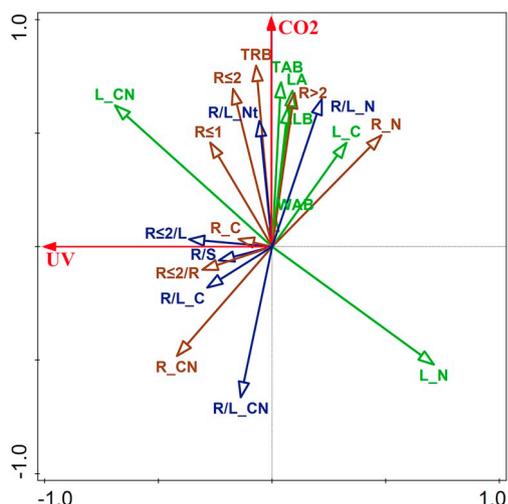
Fig. 7. Ordination diagram (biplot) of redundancy analysis (RDA) for two environmental drivers (CO_2 = CO_2 concentration and UV = intensity of UV radiation) indicated by red arrows, six aboveground traits (TAB = total aboveground biomass, LA = leaf area, LB = leaf biomass, WAB = woody aboveground biomass, L_CN = C:N ratio in leaves, L_C = C content in leaves, L_N = N content in leaves) indicated by green arrows, seven belowground biomass traits (TRB = total root biomass, R ≤ 2 = root biomass of roots with diameter ≤ 2 mm, R ≤ 1 = root biomass of roots with diameter ≤ 1 mm, R > 2 = root biomass of roots with diameter > 2 mm, R ≤ 2/R = ratio of roots with diameter ≤ 2 mm to total root biomass, R_CN = C:N ratio in roots, R_C = C content in roots, R_N = N content in roots) indicated by brown colour, and six allocation traits (R/S = root/shoot biomass ratio, R ≤ 2/L = ratio of root biomass with diameter ≤ 2 mm/leaf biomass, R/L_CN = root C:N/leaf C:N ratio, R/L_C = root C/leaf C contents ratio, R/L_N = root N/leaf N contents ratio, R/L_Nt = total root N/total leaf N ratio) indicated by blue colour. The angle of a given trait's arrow relative to any other trait's arrow indicates mutual correlation; an arrow's length indicates multiple correlation of the given trait with the ordination axes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.2. Carbon and nitrogen balance and nitrogen reallocation

C:N status of plants can be considered as one of the most relevant indicators of source:sink balance. C:N stoichiometry can regulate expression of genes involved in N acquisition and metabolism (Martin et al., 2002), secondary metabolism (Larbat et al., 2012), and photosynthesis (Paul and Foyer, 2001), as well as plant growth and biomass allocation (Hermans et al., 2006). A high C:N ratio leads to an accumulation of carbohydrates (Paul and Driscoll, 1997), which has been suggested as a main signalling and regulatory mechanism for changes in root system architecture and increases in the root-to-shoot ratio (Hermans et al., 2006). Changes in the C:N ratio induced by [CO_2] and
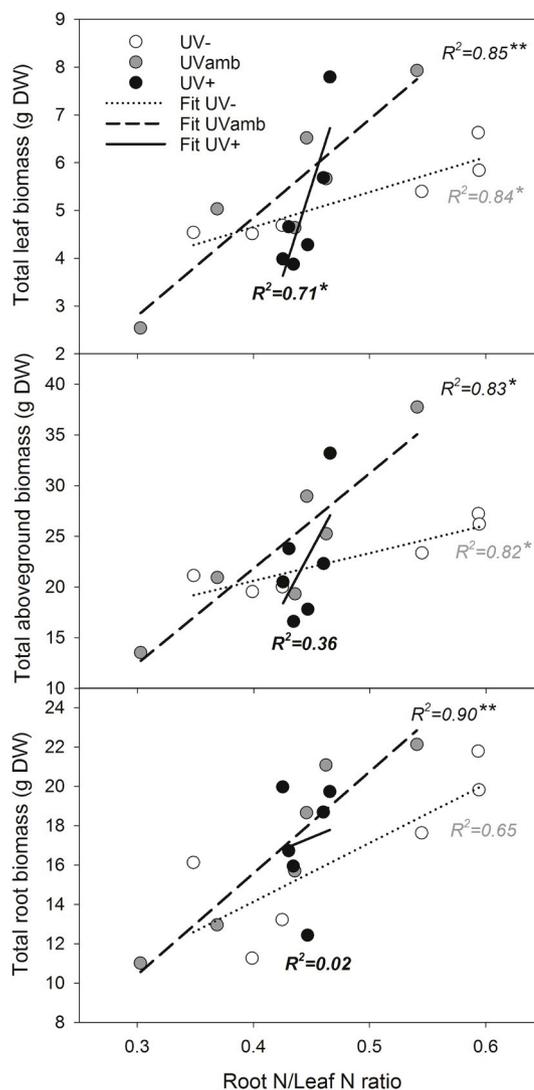
Fig. 9. Relationships between the ratio of nitrogen content in roots and leaves (Root N/leaf N ratio) and total leaf biomass (A), total aboveground biomass (B), and total root biomass (C) estimated in *Fagus sylvatica* saplings and analysed separately for each UV treatment. UV– represents exclusion of UV-B and UV-A radiation, UVamb represents ambient UV intensity, and UV + represents UV-B and UV-A radiation enhanced by 150% as compared to UVamb treatment. The means for individual replication (points) and regression lines are presented. Coefficients of determination (R^2) and statistical significance (* represents $p < 0.05$ and ** represents $p < 0.01$) are indicated for each relationship.
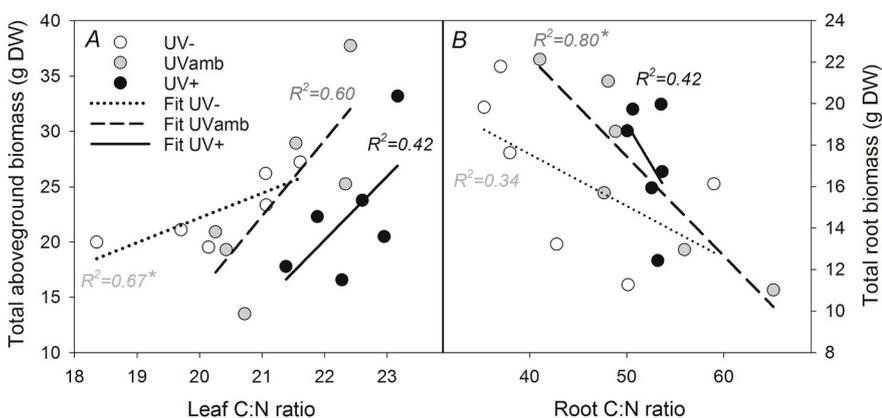
Fig. 8. Relationships between C:N ratios in leaves (**A**) and roots (**B**) and total above- and belowground biomass, respectively, analysed separately for each UV treatment. The means for individual replications (points) and regression lines are presented. Coefficients of determination (R^2) and statistical significance (* represents $p < 0.05$) are indicated for each relationship.

UV radiation may thus have a substantial effect on plant growth and biomass allocation.

Accordingly, we found significant effects of $[CO_2]$ and UV radiation on N content in leaves and roots as well as an effect on the C:N ratio. While enhanced UV radiation led to reduced N content in both leaves and roots under AC conditions, EC reduced N content in leaves but increased N content in roots. This decrease in N content in beech leaves is consistent with an observed decrease in Rubisco content, the main sink of N in leaves, under conditions of EC and enhanced UV (Urban et al., in this issue). Although a UV effect on N content and/or C:N balance is rarely reported (Björn et al., 1997), many papers have shown decreases in leaf N content per unit dry mass under elevated $[CO_2]$ (reviewed by Urban, 2003; McMurtrie et al., 2008; Leakey et al., 2009). In contrast to what is seen in leaves, such decreases of N content in FR are usually small (Leakey et al., 2009). In this study, we even found increased N content in the most actively growing FR of EC plants under UV– conditions. An interactive effect of $[CO_2]$ and UV on root N content and C:N ratio in roots was thus revealed.

A modelling study (McMurtrie et al., 2008) has shown that morphological responses to $[CO_2]$ are sensitive to the photosynthetic capacity per unit leaf N content as well as to N partitioning between aboveground and belowground biomass, and particularly to N content in leaves and fine roots. Such evidence was supported by the RDA analysis of our data (Fig. 7), which showed a strong association between the ratio of N content in roots and leaf (root N/leaf N) and $[CO_2]$, and by the relationships between root N/leaf N and biomass parameters (Fig. 9). A strong interactive effect of $[CO_2]$ and UV radiation on root N/leaf N, however, resulted in strong response to $[CO_2]$ under UV– and weak response to $[CO_2]$ under UV+ conditions. The results also show that the C:N ratio is affected differently by UV radiation and $[CO_2]$ in leaves than in roots. While elevated $[CO_2]$ increases C:N in leaves, it has an opposite effect in roots. Similarly to our results, an increase of N content and reduction of the C:N ratio has been found in roots of *Calamagrostis epigejos* under conditions of elevated $[CO_2]$ and excluded UV (Rozema et al., 1997). These changes were associated with low accumulation of lignin under reduced UV intensities.

It has been shown that C and N availability have a regulatory feedback effect on plant metabolism and development (Coruzzi and Zhou, 2001). Adaptation to changing C:N conditions includes precise partitioning of C and N sources and fine-tuning of complex cellular metabolic activity (Sulpice et al., 2013). Low C:N ratio in leaves as a consequence of elevated $[CO_2]$ treatment may lead to reduced biosynthesis of polyphenols like flavonols (Meyer et al., 2006) and thus may result in increased sensitivity of plants to photoinhibition (Klem et al., 2015). Moreover, Gargallo-Garriga et al. (2014) have shown that C:N stoichiometry influences biosynthesis of primary metabolites – saccharides and amino acids – and their allocation to roots and shoots. Such linkages between primary and secondary metabolism can explain our findings on the C:N ratio's role as an integrator of the responses to elevated $[CO_2]$ and UV radiation (Figs. 7–9), as seen through the relationships between the C:N ratio, N allocation, and biomass production. The shifts of slope and intercept of such relationships with UV intensity also indicate, however, that the role of C:N balance and N allocation is rather complex and so more-detailed knowledge at the level of metabolic profiles is needed to fully understand responses to elevated $[CO_2]$ and UV radiation.

5. Conclusions

In accordance with the hypothesis tested, our results confirmed interactive effects of CO_2 concentration and UV radiation on biomass production and particularly on biomass allocation to FR and aboveground biomass. The strongest stimulatory effect of EC on aboveground biomass and roots was found under ambient UV radiation, whereas the effect of EC was weaker at both lower and higher UV doses. Nitrogen content in the roots and the distribution of nitrogen between leaves and

roots were also significantly influenced by a combined effect of $[CO_2]$ and UV radiation. The observed changes in leaf and root C:N stoichiometry and N allocation are associated with altered morphological traits, and particularly with aboveground and FR biomass. A deeper understanding of the relationships between C:N stoichiometry, plant organ stoichiometry, and biomass accumulation is required to understand plant growth processes under conditions of elevated $[CO_2]$ and UV radiation.

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