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RESEARCH PAPER

Impact of interspecific competition and drought on the allocation of new assimilates in trees

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ABSTRACT

In trees, the interplay between reduced carbon assimilation and the inability to transport carbohydrates to the sites of demand under drought might be one of the mechanisms leading to carbon starvation. However, we largely lack knowledge on how drought effects on new assimilate allocation differ between species with different drought sensitivities and how these effects are modified by interspecific competition. We assessed the fate of ^{13}C labelled assimilates in above- and belowground plant organs and in root/rhizosphere respired CO_2 in saplings of drought-tolerant Norway maple (*Acer platanoides*) and drought-sensitive European beech (*Fagus sylvatica*) exposed to moderate drought, either in mono- or mixed culture. While drought reduced stomatal conductance and photosynthesis rates in both species, both maintained assimilate transport belowground. Beech even allocated more new assimilate to the roots under moderate drought compared to non-limited water supply conditions, and this pattern was even more pronounced under interspecific competition. Even though maple was a superior competitor compared to beech under non-limited soil water conditions, as indicated by the changes in above- and belowground biomass of both species in the interspecific competition treatments, we can state that beech was still able to efficiently allocate new assimilate belowground under combined drought and interspecific competition. This might be seen as a strategy to maintain root osmotic potential and to prioritise root functioning. Our results thus show that beech tolerates moderate drought stress plus competition without losing its ability to supply belowground tissues. It remains to be explored in future work if this strategy is also valid during long-term drought exposure.

INTRODUCTION

Current climate models predict that numerous forest regions in Central Europe will experience increasing frequencies and severities of drought periods (Christensen *et al.* 2007; IPCC 2007). The expected drier climatic conditions will not only have consequences for photosynthesis and primary productivity (Ciais *et al.* 2005; Granier *et al.* 2007), but will also affect plant carbon (C) allocation dynamics (Epron *et al.* 2012; Bahn *et al.* 2013; Zang *et al.* 2014), having major impacts on the global C balance of terrestrial ecosystems. In trees, the interplay between reduced C assimilation and the inability to transport carbohydrates to the sites of demand is presently discussed as one of the mechanisms leading to C starvation and finally tree dieback under drought (Sala *et al.* 2010).

Numerous studies have revealed a rapid and close link between photosynthesis and energy demanding processes, especially in roots and rhizosphere (Högberg *et al.* 2001, 2008, 2010; Epron *et al.* 2011), thus suggesting that functioning of the belowground compartment in forest ecosystems strongly depends on the transfer of new assimilates. Moreover, it is necessary to consider the fate of newly assimilated carbohydrates in different plant tissues as well as the long-distance transport *via* the phloem (Paterson *et al.* 2009; Ruehr *et al.* 2009; Offermann *et al.* 2011) to understand the biomass allocation dynamics as well as root functioning in plants under changing environmental conditions (Blessing *et al.* 2015).

It is widely acknowledged that new assimilates are preferably transferred to tissues with the highest C demand (Lambers *et al.* 2008). For example, under light limitation, plants tend to transport larger amounts of photosynthate to aboveground organs, whereas under reduced nutrient availability or drought they can allocate more C to the root system (Bloom *et al.* 1985; Kobe *et al.* 2010; Poorter *et al.* 2012; Brunner *et al.* 2015). In their review, Poorter *et al.* (2012) indicate that moderate water stress only leads to a small increase in the root mass fraction

(*i.e.* the relative contribution of root biomass to total biomass) of plants, and thus only to a slight increase in belowground C allocation. Under intense drought, in contrast, the transport of recent assimilates belowground strongly decreased in drought-sensitive European beech under severe drought (Ruehr *et al.* 2009). Moreover, Meier & Leuschner (2008a) report a strong reduction in fine root biomass of European beech under reduced soil water supply, not only due to a shorter fine root lifespan but also to a ten-fold reduction in growth rates. Thus, there seems to be no unequivocal plant C partitioning and assimilate allocation response to drought, and more information is necessary to understand allocation priorities and mechanisms in plants under environmental stress.

Pulse labelling approaches, applying either ^{13}C- or ^{14}C-enriched CO_2, allow tracing of the allocation of recent assimilates to respiration, growth and storage pools (Kagawa *et al.* 2006; Carbone *et al.* 2007; Epron *et al.* 2012; Hartmann *et al.* 2015) and thus are highly suitable tools to assess the mechanistic basis of short-term C partitioning in plants and between plants and the rhizosphere.

There is some information on how changing environmental conditions, such as reduced water availability, affect these short-term dynamics of C fluxes and patterns of assimilate distribution within trees (*e.g.* Ruehr *et al.* 2009; Savage *et al.* 2015). For the rather drought-sensitive European beech, soil water restriction not only decreases the amount of new assimilates transferred belowground but also increases transport times considerably (Barthel *et al.* 2011; Zang *et al.* 2014). Recent reviews describe drought effects on phloem physiology in plants and conclude that reduced phloem transport, as well as phloem failure with restricted water supply, could occur either because of viscosity build-up at the source sites or a failure to maintain phloem water status and cell turgor (Sevanto 2014; Savage *et al.* 2015). Moreover, phloem loading might be reduced due to decreased transport of assimilates in the mesophyll, resulting from tissue dehydration (Van Bel & Gamalei 1992) as well as down-regulation of the expression of sucrose transporters under osmotic stress (Pommerrenig *et al.* 2007). Nevertheless, we largely lack knowledge on how drought effects on assimilate allocation differ between species with different drought sensitivity, and how they are modified by inter- *versus* intraspecific competition. It is generally accepted that interspecific competition can result in changes in biomass allocation to tree fine roots, leading either to a reduction of fine root biomass (Brandtberg *et al.* 2000; Schmid & Kazda 2002) or to increased fine root growth (Leuschner *et al.* 2001), depending on the competitive abilities of the species involved. There is, however, no information on how interspecific competition changes short-term sink priorities as assessed by tracing isotopically labelled assimilates.

The biosphere mediates the C flow from the atmosphere to the soil system also on short time scales (Barthel *et al.* 2011; Blessing *et al.* 2015), and in future the frequency of extreme events such as drought will most likely increase. It is thus important to understand the mechanisms that drive C allocation, C transfer times and above-to-belowground coupling strength as affected by environmental drivers. Moreover, forestry in Central Europe, as well as in many other regions worldwide, promotes mixed species stands that are assumed to be more resistant to environmental stressors (Millar *et al.* 2007; Pretzsch *et al.* 2013) and to provide higher levels of multiple ecosystem services (Gamfeldt *et al.* 2013). As a consequence, insights concerning the effects of not only abiotic drivers but also species interactions on mechanisms that control C allocation within trees are crucial.

The present study aims to increase understanding of the plant physiological mechanisms affecting key parameters of carbohydrate assimilate transport in trees under water restriction and interspecific *versus* intraspecific competition. We assessed the fate of ^{13}C-labelled assimilates in above- and belowground plant organs and in root/rhizosphere respired CO_2 in two tree species. Saplings of Norway maple (*Acer platanoides* L.), known to be rather drought-tolerant (Linder 2000; Webster *et al.* 2005) and European beech (*Fagus sylvatica* L.), assumed to be sensitive to reduced water availability (Gessler *et al.* 2007), were exposed to restricted soil water supply either in mono- or in mixed cultures. We exposed the trees to moderate drought as normally expected in the forest understorey, in which extreme conditions (particularly soil moisture, air humidity and temperature) are normally buffered *via* the overstorey canopy (Fotelli *et al.* 2003).

We hypothesised that (i) drought would in general lead to a clear reduction of assimilate transport belowground due to impairment of phloem transport. We thus expected a lower ^{13}C signal in soil respired CO_2 (*i.e.* in CO_2 mainly originating from root/rhizosphere respiration). Furthermore, we expected a clear drought-mediated increase in the mean residence time (MRT) of new assimilates in leaves and a delayed appearance of the ^{13}C label in root organic matter and soil respiration. We hypothesised these effects to be stronger in the drought-sensitive beech compared to the more drought-tolerant maple. Moreover, we assumed that (ii) in the mixed species treatment, maple would be able to out-compete the drought-sensitive beech under water restriction. With beech assimilate transport impaired by drought, maple should be able to better take advantage of limited water resources by increasing belowground C allocation.

MATERIAL AND METHODS

Plant material and experimental set up

The study was conducted in a greenhouse with controlled air temperature (20 ± 2 °C). To simulate a diurnal cycle with a light period of 15 h, additional artificial illumination was applied (bulb type NARVA NC 1000–00) and the photosynthetic photon flux density (PPFD) at canopy level was not $<600\ \mu mol \cdot m^{-2} \cdot s^{-1}$. Two different tree species typical for mixed deciduous forests in Central Europe were selected for the experiment. *Fagus sylvatica* L. (European beech) is the most abundant species of the potential natural vegetation in Central Europe. Because of its sensitivity to low water availability (Ellenberg 1996) and longer drought periods (Fotelli *et al.* 2002), physiological performance, growth and competitive ability of European beech may be adversely affected under future climatic conditions (Rennenberg *et al.* 2004). *Acer platanoides* L. (Norway maple) has a natural distribution area spanning from Scandinavia and Russia to southern France and Turkey. It is known for high water-use efficiency (WUE; Kloeppel & Abrams 1995; Hommel *et al.* 2014) and is considered to be relatively drought-tolerant (Linder 2000).

Tree saplings were obtained from a commercial tree nursery (Handel, Metzingen, Germany). The 2-year-old seedlings were planted in 60-l plant tubs filled with organic, peaty substrate (Kausek, Mittenwalde, Germany) before leaves developed. We applied 50 g lime (Rüdersdorf, Germany) per tub. For all competition treatments, ten tree individuals were planted in a tub and the average distance between individuals was *ca*. 5–10 cm. Three competition treatments were established by planting (i) Norway maple in monoculture (10 plants per tub); (ii) European beech in monoculture (10 plants per tub); and (iii) Norway maple and European beech in mixed culture (five individuals of each species per tub; randomly planted; Figure S1). The monocultures thus had the same number of trees and planting density as the mixed cultures. There were 20 tubs per competition treatment, resulting in 200 trees per treatment, thus 600 trees in total.

The bulk density of the organic soil substrate was 0.12 ± 0.03 g·cm^{-3}. Volumetric soil water content (θ_s) was monitored continuously with EC-5 soil moisture sensors (Decagon Devices, Pullman WA, USA) at a depth of 10–15 cm (Table 1). Soil water potential (ψ_s) was derived from θ_s (%) according to Schindler *et al.* (2010). During the acclimation period in the greenhouse, the tubs with plants were watered every second day to field capacity. After 3 months of growth in the greenhouse, half of the tubs of each competition treatment (30 tubs in total) were exposed to moderate soil water restriction (drought treatment). The drought treatment started after the leaves had fully developed.

We lowered ψ_s to the root water uptake thresholds of soil moisture tension (-0.21 to -0.24 MPa) for forest tree species (Bittner *et al.* 2010) and these thresholds were reached after a couple of days without watering. When ψ_s decreased below this threshold, the tubs were carefully watered to reach again the target values. The achieved thresholds in soil moisture tension allow a minimum root water uptake as defined in Bittner *et al.* (2010); below the threshold value, the root water uptake is assumed to be zero. By adjusting ψ_s to the minimum water uptake threshold, we exposed plants to moderate drought but avoided damage or death due to severe drought conditions (Hommel *et al.* 2014). After the pulse labelling (see below), plants were no longer watered to avoid variability in soil moisture tension, which might have affected the label distribution.

The other half of the tubs was well watered by maintaining soil moisture at field capacity (control treatment). On day 14 of the drought treatment, the plants were pulse labelled with $^{13}CO_2$. θ_s was between 44% and 50% in the controls and between 26% and 33% at the end of the drought treatment (on day 18 after the onset of drought, equivalent to day 4 after $^{13}CO_2$ labelling).

Determination of $\delta^{13}C$ in soil CO_2

For each treatment, 7.0 cm × 2.5 cm sintered glass diffusers (Antstore Europe, Berlin, Germany) with gas-permeable porous walls were installed before planting the trees horizontally at depths of 20–25 cm into the soil. The diffusers were connected

Table 1. Characterisation of the species and the experimental conditions.

	monocultures		mixed culture	
species	maple	beech	maple	beech
age (years)	2	2	2	2
height (cm)	40–80	30–50	40–80	30–50
θ_s (%)				
control	47.9 ± 0.1	43.8 ± 2.0	50.0 ± 0.1	
drought	33.0 ± 0.1	25.6 ± 3.0	27.7 ± 0.1	
leaf biomass (g)	Species: $P < 0.01$; Drought: n.s; Competition: $P < 0.01$; Species x Competition: $P = 0.02$			
control	2.77 ± 1.99	4.33 ± 1.88	9.84 ± 4.42	1.69 ± 0.60
drought	4.75 ± 2.51	3.11 ± 0.85	5.28 ± 3.44	1.53 ± 0.56
stem biomass (g)	Species: $P < 0.01$; Drought: n.s; Competition: $P = 0.03$; Species x Competition: $P < 0.01$			
control	4.84 ± 1.63	8.97 ± 3.45	15.09 ± 9.87	4.07 ± 1.71
drought	5.59 ± 2.87	5.29 ± 1.73	9.43 ± 5.34	3.07 ± 1.47
root biomass (g)	Species: $P < 0.01$; Drought: n.s; Competition: n.s.; Species x Competition: $P < 0.01$			
control	3.38 ± 1.85	8.81 ± 4.41	5.75 ± 2.82	4.91 ± 2.08
drought	2.66 ± 1.36	7.97 ± 4.82	6.01 ± 3.46	4.75 ± 1.30
R:S ratio				
control	0.44	0.66	0.23	0.85
drought	0.25	0.95	0.41	1.03
harvests	6	6	4	4

Plant age is given in years and the leaf, stem and root biomass in gram (g) dry weight per plant. Tree height at the beginning of the experiment and number of harvest times in each scenario is also given. At each harvest, three plants per treatment and species were sampled. θ_s is the average volumetric water content (±SD) of the control (n = 6) and drought (n = 6) treatment during the whole drought treatment period, R:S ratio is the root:shoot ratio. Leaf, stem and root biomass is given as average of the treatment (control/drought) of all trees at all harvest time points (monocultures n = 18, mixed n = 12). A statistical difference in plant biomass was assessed by GLM ANOVA. n.s.: not significant.

with gas-tight PTFE tubing to transfer the soil gas *via* a multiport Valco valve sequencer (Vici, Houston, TX, USA) to a cavity ring-down isotopic laser spectrometer (G2101-i Picarro Isotopic CO_2 Analyzer; Sunnyvale, CA, USA). Flow rates for all tubes were adjusted *via* pumps to 100 $l \cdot h^{-1}$ controlled by flow meters and needle valves. The soil gas $^{12}CO_2$ and $^{13}CO_2$ concentrations (and thus the $\delta^{13}C$) were sequentially measured for all six tubs. Each tub was connected for 50 min to the laser spectrometer, followed by measurement of the CO_2 concentration of the ambient air for 10 min. The laser spectrometer was calibrated before and after the experiment. The drift was <1‰, which is negligible compared to the strong ^{13}C enrichment achieved due to the labelling.

The $^{13}CO_2$ pulse labelling

At 18 h prior to the $^{13}CO_2$ labelling, the belowground compartment was separated and made gastight from the aboveground compartment by coating the soil surface with liquid silicone (TACOSIL 145; Dresden, Germany). We drilled holes in the sidewall of the tubs to prevent anaerobic conditions in the soil compartment. Prior to labelling, transparent chambers (0.48 m × 0.74 m × 1.5 m; volume *ca.* 530 l) were placed over each tub and sealed gas tight. The $^{12}CO_2$ concentrations within six chambers were measured simultaneously with portable gas analysers (IRGA; Li-820; LiCor, Lincoln, NE, USA).

A beaker filled with phosphoric acid (H_3PO_4) was placed inside each chamber. A 99% ^{13}C sodium bicarbonate label was dissolved in distilled water (0.3 g label per tub) and injected into the beaker with acid to release $^{13}CO_2$ as soon as the CO_2 concentration inside the chamber approached *ca.* 100 ppm due to the plant CO_2 uptake during photosynthesis. Within a few minutes, the $^{13}CO_2$ released yielded a CO_2 concentration of 310 ppm within the transparent chamber. Plants were thus exposed to *ca.* 410 ppm CO_2 (*ca.* 310 ppm $^{13}CO_2$, and *ca.* 100 ppm $^{12}CO_2$) at the beginning of the labelling experiment. Two hours after the start of labelling, the chamber and the silicon layer separating the soil and the aboveground compartment were removed. During labelling, the air temperature increased by no more than 5 °C in all treatments.

Harvest and isotope analyses

Plants were harvested after 2 h (immediately at the end of labelling), 24 h, 48 h and 96 h after the start of the $^{13}CO_2$ labelling. At each harvest time point, three plants per treatment and species were sampled from different tubs. Furthermore, assimilation rate (A), transpiration (E) and stomatal conductance (g_s) were measured with a portable infrared gas exchange analyser (GFS-3000; Walz, Effeltrich, Germany). Whole plant transpiration (*i.e.* transpiration rate of an individual beech or maple) was up-scaled from leaf level transpiration *via* whole plant leaf biomass and specific leaf area (van Hees 1997; Barthod & Epron 2005).

In order to avoid disturbing the $^{13}CO_2$ soil gas analyses through plant harvest, we excluded six of the 20 tubs (three drought, three control) from each competition treatment regarding the destructive harvest. These six excluded tubs were only used for the online CO_2 measurements, whereas the remaining 14 tubs were used only for plant harvest. Each harvested plant was separated into leaves, upper phloem (3 cm below the upper end of the main stem) and associated xylem, lower phloem at the stem base (3 cm above soil surface) and associated xylem and roots. Total biomass of the different fresh plant organs was determined directly after harvest, and an aliquot was dried at 75 °C in an oven until the mass was constant to determine dry weight.

A subset of the harvested plant material was placed in plastic tubes and immediately frozen in liquid nitrogen and stored at −30 °C. Thereafter, these samples were ground in liquid nitrogen and water soluble organic matter (WSOM) was extracted for analysis of the C isotopic composition and C content according to Ruehr *et al.* (2009) and Gessler *et al.* (2009). For WSOM extractions, 70 ± 5 mg leaf, phloem and xylem fresh material, and 140 ± 5 mg fresh root material was used.

Briefly, ground plant material was shaken at 4 °C with deionised water for 1 h using an incubator (Enviro Genie SL1200; Enviro Genie, Scientific Industries, Inc., Bohemia, NY, USA). The samples were then heated (Thermomixer, Eppendorf, Germany) to precipitate soluble proteins, and centrifuged twice. An aliquot of each supernatant was transferred to tin capsules and dried at 30 °C (Concentrator Plus, Eppendorf, Germany). $\delta^{13}C$ in the dried extracts was determined by combusting the samples in a Flash HT elemental analyser (ThermoFinnigan, Bremen, Germany) coupled *via* a ConFlo III interface to a Delta V Advantage isotope ratio mass spectrometer. The precision of the measurement was <0.10‰. The isotope values are expressed in delta notation (‰ units), relative to VPDB (Vienna Pee Dee Belemnite).

To estimate the amount of ^{13}C added through pulse labelling to the WSOM pool of the leaves and roots, $\delta^{13}C$ values were converted into atom% ^{13}C. We then calculated the ^{13}C excess, *i.e.* the increase in amount of ^{13}C of the WSOM pool due to the $^{13}CO_2$ exposure, according to Ruehr *et al.* (2009), based on the total leaf and root biomass of a single plant for the time point of maximum ^{13}C incorporation in the given tissue. We computed the mean residence time (MRT) of WSOM in leaves and for soil $^{13}CO_2$. We acknowledge that besides transport processes in the plant, differences in soil diffusivity in the different treatments might have affected the temporal pattern of the ^{13}C label in soil CO_2. Since we collected the CO_2 directly in the rooting zone, we assumed this effect to be negligible. The mean residence time corresponds to the C stock to C flux ratio (Epron *et al.* 2012) and was calculated by fitting the following exponential decay function.

$$N(t) = N_0 e^{(-\lambda t)} \quad (1)$$

where t is time in hours after labelling; N_0 is initial quantity of $\delta^{13}C$ at time t = 0 (^{13}C peak); λ is the decay constant; and N(t) is quantity of ^{13}C after time t. The mean residence time (in hours) was then calculated as MRT = 1/λ.

Statistical analyses

All measured variables (in the drought and the control treatment) were tested for normality and characterised using descriptive statistics (means and SD). Effects of species, drought treatment and competition treatment on biomass, photosynthesis, stomatal conductance, ^{13}C excess and MRT for leaf WSOM were assessed with GLM ANOVA. For the analyses,

individual species were nested within the competition treatment. To compare the time courses of $\delta^{13}C$ in WSOM of different tissues, we applied a factorial, repeated measures ANOVA where individual species were nested in the competition treatment. For $\delta^{13}C$ in soil CO_2, we only compared drought and well-watered control for each treatment (beech monoculture, maple monoculture and beech–maple mixed culture) with repeated measures ANOVA since the origin of CO_2 could not be distinguished between the two species in the mixed treatment. MRT for soil CO_2 of drought and well-watered control were compared using Student's *t*-test.

The error bars in the figures represent ±SD. Statistical analyses and fits were carried out with R 2.8.0 (R Development Core Team 2010; Pinheiro *et al.* 2008), NCSS 2004 (Number Cruncher Statistical Systems, Kaysville, UT, USA) and SIGMAPLOT 12.3 (Systat Software, Inc., San Jose, CA, USA).

RESULTS

Biomass analyses and plant gas exchange measurements

Leaf biomass per individual plant for the two monocultures and the mixed culture was not significantly affected by the drought treatment (Table 1). Competition affected leaf biomass and a significant interaction between species and competition was observed, leading to greater mass in inter- compared to intra-specific competition in beech and opposite patterns in maple. There was no significant effect of drought on stem and root biomass. For stem biomass, a species and competition effect and their interaction was detected. Comparable to leaves, stem and root biomass increased under interspecific competition in maple and decreased in beech. Drought decreased root to shoot ratios of maple in monoculture, while it increased in beech. In the mixed cultures, root to shoot ratios increased upon drought in both species.

A was significantly reduced by drought. Moreover, a species effect was detected and interspecific competition led to a general reduction of A compared to intraspecific competition. While the average assimilation rate of maple did not differ between mono- and mixed cultures under well-watered control conditions, amounting to 4.5 ± 2.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the observation period (Fig. 1), beech rates were slightly lower in the mixed culture (5.1 ± 1.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to monocultures (7.5 ± 2.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$). As observed for A, g_s was reduced by drought and g_s values were generally lower in maple compared to beech (Fig. 1). Whole plant transpiration (E_{plant}) was different among species and showed a species and competition interaction with beech showing lower and maple higher transpiration rates in the mixed cultures compared to the monocultures (Figure S2). Moreover, drought significantly reduced transpiration for both species across all competition treatments.

The $\delta^{13}C$ in soil respired CO_2

We followed the time course of the $\delta^{13}C$ signal in soil respired CO_2 after the label pulse to assess the intensity and the speed of the linkage between photosynthesis and energy demanding processes belowground (Kayler *et al.* 2010). We acknowledge that the CO_2 derived from the soil is a mixture originating from autotrophic and heterotrophic respiration; the increase in the ^{13}C signal after pulse labelling, however, can be mainly attributed to root and rhizosphere respiration. All $\delta^{13}C$ values in the extracted soil CO_2 before label application were below −20‰ and thus well within the range observed for soil respiration (Werner & Gessler 2011) indicating that dilution of soil respired CO_2 by ambient atmospheric CO_2 was negligible.

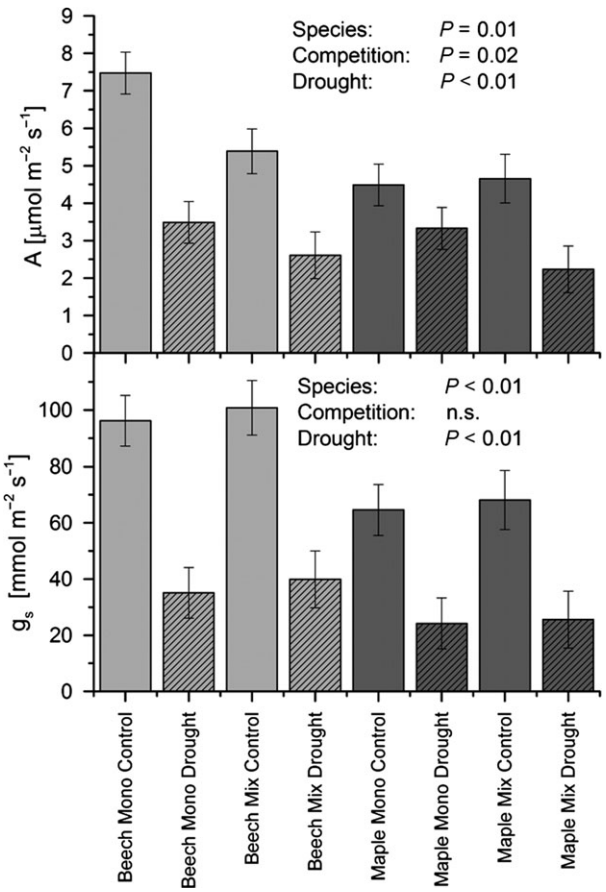

Fig. 1. Assimilation rate (A) and stomatal conductance (g_s) of beech and maple in the drought and competition treatments. Values given are averages from gas exchange measurements 24 h before the ^{13}C labelling and 2, 24, 48 h after labelling. Measurements were taken from three replicates from the drought and control treatments for monocultures and the mixed culture.

The first increase in $\delta^{13}C$ after labelling, i.e. the first appearance of ^{13}C tracer was detected in all water availability and competition treatments for beech and maple after approx. 10 h (Fig. 2). It is evident from Fig. 2A that drought had neither a clear effect on the peak height nor on the slope of the curve in maple grown in monoculture. There were no significant differences in $\delta^{13}CO_2$ as tested with repeated measure ANOVA between drought exposed and control trees and the MRT was unaffected by drought (Table 2). Nevertheless, the $\delta^{13}CO_2$ signal peaked earlier under drought compared to the well-watered treatment, and already decreased when $\delta^{13}CO_2$ was still increasing until approx. 26 h in the control.

In beech monocultures, drought significantly decreased the amount of ^{13}C label that arrived in the soil CO_2 (Fig. 2B). MRT in the beech monoculture control treatment was approx. five-fold higher compared to the maple control. There was a tendency for MRT to increase upon drought in beech monocultures, but the difference between treatments was not

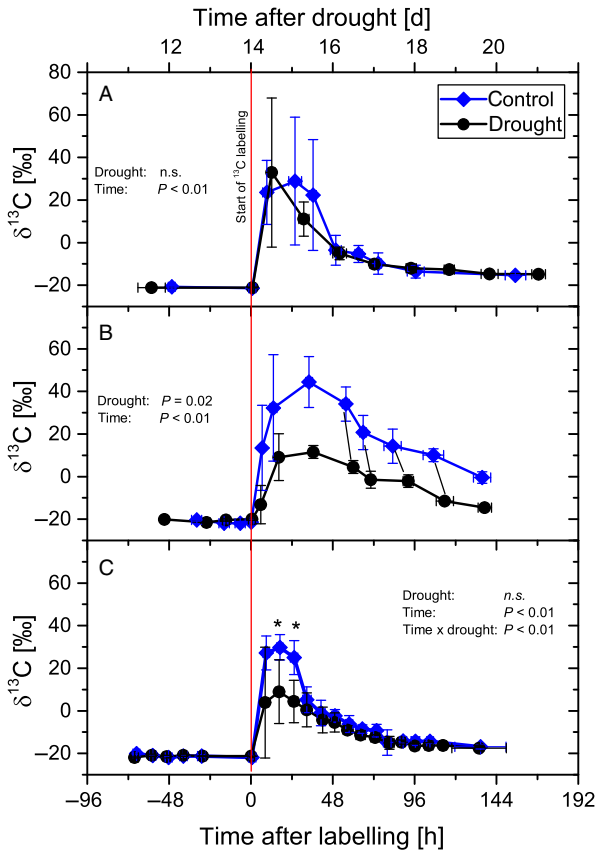

Fig. 2. $\delta^{13}C$ in soil CO_2 after canopy ^{13}C label application. Blue symbols and lines indicate the control, and black symbols and lines drought treatments. The red line indicates the start of pulse labelling. $\delta^{13}C$ values of soil respired CO_2 in maple monoculture (A), beech monoculture (B) and mixed culture of maple and beech (C) are displayed. $\delta^{13}C$ in soil CO_2 was compared between drought and control for the different intra- and interspecific competition treatment by applying repeated measures ANOVA. n.s.: not significant. In (C) an additional test was carried out: the three-first time points after labelling were compared (drought *versus* control) by Student's *t*-test; * denotes significant differences ($P \leq 0.05$). In (B), the four black ledger lines indicate which time points were compared since there was a slight time shift between measurements of drought and control tubs. Horizontal and vertical error bars indicate SD.

significant (Table 2). The label concentration arrived and peaked almost simultaneously in the control treatments, *i.e.* within 34–38 h and thus slightly later than in maple.

In the mixed culture, there was a tendency for a reduction in $\delta^{13}C$ of soil CO_2 compared to monocultures. This is indicated by the significant interaction between (sampling) time and drought. Furthermore, at two time points a significant reduction of $\delta^{13}C$ upon drought was observed (Fig. 2C). Mean residence time remained unaffected by drought and was in-between the values for the two monocultures (Table 2). The label maximum in CO_2 was detected after approx. 16 h with no difference between the drought and the control treatment.

The $\delta^{13}C$ of WSOM in the different plant organs

Leaves

The maximum $\delta^{13}C$ in leaf WSOM for both species in all treatments always occurred directly after the termination of labelling (equalling 2 h after labelling onset; Fig. 3). In the monocultures of maple, there was a slight decrease in $\delta^{13}C$ of leaf WSOM upon drought at all harvest dates whereas beech showed the opposite pattern. However, neither drought nor species affected $\delta^{13}C$, but $\delta^{13}C$ values were significantly lower for the interspecific competition treatment. For ^{13}C excess at the individual plant level (Table 2) that was determined in the foliage directly after labelling, there were significant effects of species and competition treatment but no direct effects of drought. The somewhat smaller ^{13}C excess in drought treated monoculture beech compared to controls is explained by the slightly smaller total leaf biomass (Table 1).

The MRT was not affected by species, drought or competition. In beech, however, the mixed culture MRT decreased under drought and increased under well-watered control conditions compared to the monocultures (Table 2) whereas the opposite was found for maple. This is indicated by the statistically significant interaction between species, drought and competition.

Phloem and xylem

The $\delta^{13}C$ in WSOM peaked 2 h after labelling in the upper stem phloem of all treatments with the exception of the maple in mixed culture under drought (Fig. 3). In the upper phloem, there was a significant competition effect with higher values in monocultures and a species effect indicating higher $\delta^{13}C$ in maple compared to beech. In the lower phloem, the label peak was shifted towards 24 h (with the exception of well watered maple in monoculture) and a species effect was not found afterwards. However, the competition effect was preserved.

In the upper and lower stem xylem, the $\delta^{13}C$ peak occurred mostly after 24 h, and there was no significant drought effect observed. In contrast, a significant competition effect indicated lower $\delta^{13}C$ values in the mixed compared to the monoculture treatments.

Roots

The $\delta^{13}C$ in root WSOM peaked after 24 h in both species, independent from drought or competition treatment (monoculture *versus* mixed culture) (Fig. 3). As in the phloem and xylem, drought did not have a direct significant effect on the temporal patterns of $\delta^{13}C$ in roots but a competition effect was visible. There was however, a significant interaction between drought and species. In both, monoculture and mixture, $\delta^{13}C$ in beech was higher under drought compared to the well-watered controls whereas such a pattern could not be observed in maple.

The ^{13}C excess on a per plant basis after 24 h (when the highest label in roots was detected) showed patterns comparable to $\delta^{13}C$ in WSOM with clearly higher values in beech roots in mixed cultures as a consequence of drought (Table 2). When relating the maximum ^{13}C excess in roots (after 24 h) to the maximum excess in leaves (after 2 h), the relative amount of ^{13}C transferred to the roots clearly increased for beech in monoculture as a consequence of drought (from 15% in the control to 28% in the drought treatment) and remained more or less constant for maple (5–6%). In the mixed cultures the relative amount of ^{13}C transferred to the roots clearly increased upon drought for both species (maple: 5–46%; beech: 24–72%).

Table 2. ^{13}C excess and mean residence times. ^{13}C excess ($\mu g \cdot plant^{-1}$) is based on leaf and root biomass per average plant.

	monocultures		mixed culture	
species	maple	beech	maple	beech
^{13}C excess in leaves at ^{13}C peak	Species: $P = 0.02$; Drought: n.s.; Competition: $P < 0.01$			
control	306.9 ± 116.4	413.9 ± 296.3	196.3 ± 85.2	32.5 ± 18.2
drought	263.1 ± 174.1	219.5 ± 62.1	36.8 ± 4.5	36.3 ± 11.7
^{13}C excess in roots at ^{13}C peak	Species: $P < 0.01$; Drought: n.s.; Competition: $P < 0.01$; Drought x Competition: $P < 0.01$			
control	19.3 ± 18.9	62.9 ± 37.7	9.8 ± 8.5	7.9 ± 5.6
drought	12.8 ± 6.8	61.5 ± 35.4	16.8 ± 4.1	26.2 ± 25.9
MRT in ^{13}C WSOM leaves	Species: n.s.; Drought: n.s.; Competition: n.s.; Species x Drought x Competition: $P = 0.01$			
control	34.6 ± 12.1 h	17.3 ± 3.1 h	12.4 ± 1.2 h	28.5 ± 0.4 h
drought	25.5 ± 8.6 h	28.3 ± 5.2 h	55.1 ± 23.9 h	8.3 ± 2.1 h
MRT of $\delta^{13}C$ of soil CO_2				
control	14.1 ± 4.4 h[a]	72.5 ± 33.6 h[a]	27.3 ± 3.8 h[a]	
drought	17.1 ± 1.7 h[a]	208.3 ± 110.4 h[a]	56.5 ± 22.6 h[a]	

^{13}C excess values are given for the time point of maximum label in the given tissue. For leaves it was the harvest 2 h after start of the ^{13}C labelling, for roots it was the harvest 24 h after labelling (cf. time courses in Fig. 3). Mean residence time (MRT) is given in (h). Effects of species, drought and competition treatment as well as significant interaction as assessed with factorial ANOVA are given for tissues-specific values. Mean residence times for soil CO_2 of drought and well-watered control were compared by Student's *t*-test. Here, same letters (a) indicate no significant differences.

DISCUSSION

The effect of moderate drought on allocation of new assimilates in monocultures

Based on recent studies on ^{13}C allocation (Ruehr *et al.* 2009; Barthel *et al.* 2011; Zang *et al.* 2014), we hypothesised that drought would lead to a clear reduction in assimilate amount transported belowground and to a decrease in transport velocity in both species assessed here – but with a stronger effect for the drought-sensitive beech. In contrast to the abovementioned studies, the drought intensity in our study was rather moderate as we aimed to simulate the conditions in the forest understorey where extreme environmental amplitudes are buffered by the sheltering canopy (Hommel *et al.* 2014; Felsmann *et al.* 2015; Gimbel *et al.* 2015), and thus we kept soil moisture tension close to but above the root water uptake thresholds.

As expected, the moderate drought decreased assimilation rate in both species in monoculture, but the decrease in assimilation rate was not reflected in the ^{13}C label found in WSOM that is assumed to be a reliable proxy for newly assimilated sugars (Brandes *et al.* 2006). Moreover, the ^{13}C excess in leaf WSOM, as a measure of the total uptake and conversion of $^{13}CO_2$ to soluble sugars, was also not affected by the drought treatment. This is in agreement to the findings of Blessing *et al.* (2015), but contradicts the observations of Ruehr *et al.* (2009) for beech. The latter authors found clearly higher maximum $\delta^{13}C$ and ^{13}C excess in leaf WSOM in controls compared to drought-treated plants directly after labelling. They, however, also showed that 2 days after the ^{13}C label application, this difference had disappeared and they attributed their finding to more efficient and faster assimilate export from the leaf to the phloem under non-limited water control conditions. In our study, the maximum $\delta^{13}C$ value in leaf WSOM for both species was already detected at the first harvest, 2 h after the start of labelling, i.*e.* directly after the ^{13}C exposure had stopped. We thus might speculate that at this time, significant amounts of ^{13}C-labelled assimilates were already exported from the leaves (*i.e.* during the 2 h of ^{13}C exposure) and that this – undetected amount – might have been larger for plants in non-limited soil water conditions of the control treatment. This assumption is partially supported by the faster increase in $\delta^{13}C$ in stem upper and lower phloem in maple controls compared to drought-treated plants in monocultures, and by the higher leaf WSOM MRT in beech in the monoculture treatment, indicating generally higher turnover rates of new assimilates under well-watered control conditions.

Additionally, the putative mismatch between the reduced assimilation rate, but comparable amounts of ^{13}C label in leaf soluble sugars in the drought compared to the control treatment, could be explained by the faster use of new assimilates for storage and growth under well-watered conditions. As the leaves of both species were fully developed, growth might play a minor part. However, starch accumulation has been observed to be under environmental control, and the division of assimilates between starch and sucrose is assumed to be internally regulated to suit environmental conditions (Zeeman *et al.* 2007). It has also been observed that starch accumulates when the use of newly produced triose phosphates from the chloroplast becomes limiting to C assimilation rate, especially when photosynthesis and stomatal conductance are high (Beck & Ziegler 1989). Conversely, we might assume that the reduced photosynthesis observed in our drought treatment, for both species, might have led to less leaf starch accumulation compared to the controls. In agreement with this assumption, Barthel *et al.* (2011) showed that the mean residence time of ^{13}C-labelled sugars in plants depends on the assimilation rate,

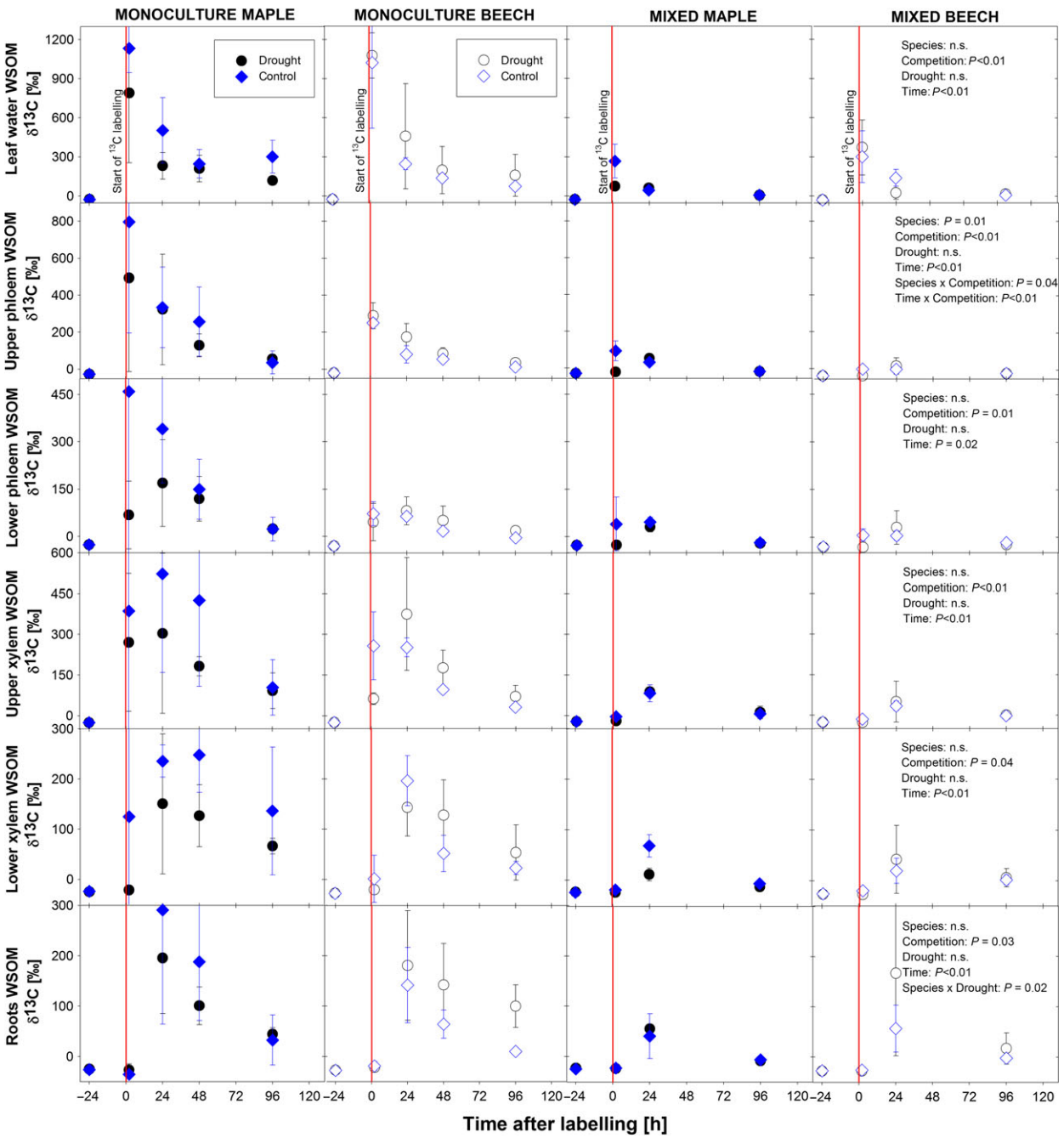

Fig. 3. Dynamics of $\delta^{13}C$ in water-soluble organic matter (WSOM) of different plant compartments in beech and maple. The four columns of graphs show beech and maple in different competition treatments (monoculture, mixed) and the water availability treatments are indicated for a given competition treatment with different colours (blue: control; black: drought treatment). The different rows of figures (from top to bottom) show $\delta^{13}C$ dynamics of WSOM in leaves, upper phloem, lower phloem, upper xylem, lower xylem and roots. The effects of species, drought and competition treatment were assessed by repeated measure ANOVA. The red line in each graph indicates the start of ^{13}C label application. Note that the y-axis scaling is different for different plant organs.

in which higher assimilation rates result in faster turnover rates, including the C utilised for respiration and storage.

While under drought, $\delta^{13}C$ values in phloem, xylem and root WSOM of maple tended to decrease, the opposite was observed for beech. For both species, we did not observe a clear time lag in label transport as a consequence of drought, neither when considering WSOM nor soil CO_2. While the first appearance of the label in soil CO_2 in the controls was comparable to results for beech and other tree seedlings (Barthel *et al.* 2011; Epron *et al.* 2012; Studer *et al.* 2014; Blessing *et al.* 2015), the absence of transport retardation upon drought was in contrast to previous findings (Ruehr *et al.* 2009; Barthel *et al.* 2011; Zang *et al.* 2014). Delays in assimilate transport from the leaves to roots for beech exposed to drought have been documented

to range up to approximately six-fold compared to controls (Ruehr *et al.* 2009). Moreover, these authors observed higher $\delta^{13}C$ values in root WSOM in the controls compared to drought-exposed beech seedlings during the first 96 h after ^{13}C exposure. Our findings, together with the previous findings of Ruehr *et al.* (2009), indicate that both drought intensity and duration strongly affect assimilate allocation in trees. In the work of Ruehr *et al.* (2009), soil water content during the drought treatment was reduced to *ca.* 30% of well-watered controls, whereas in our study, the reduction varied between 56% and 69%. Moreover, Ruehr and co-authors applied the drought for 55 days, while the drought period of the present study lasted for 2 weeks. As a consequence, the tree biomass, especially root biomass, decreased in the previous study whilst in our present study biomass was not affected. Under the severe and longer-lasting drought as applied by Ruehr *et al.* (2009), transport to belowground tissues was reduced and retarded. While under moderate drought conditions as applied in our study, transport of new assimilates was only slightly reduced (maple) or even slightly increased (beech), and no reduction in transport velocity was found. Under moderate drought, an increase in C allocation belowground might be important to maintain root functioning, since reduced water availability increases fine root mortality and simultaneously stimulates compensatory fine root production (Meier & Leuschner 2008b). However, drought intensity seems to modify the ability of the trees to allocate new assimilates to roots, confirming previous observations that severe drought can strongly retard root growth while moderate drought can enhance C allocation to roots (Eghball *et al.* 1993).

In maple, drought did not affect the amount of ^{13}C label in soil CO_2 or significantly change MRT in monoculture. This finding complements the unchanged MRT for leaf WSOM and the lack of significant direct drought effects on the $\delta^{13}C$ of WSOM in all plant organs. In contrast, beech exhibited significantly lower $\delta^{13}C$ values in soil CO_2 and a 2.9-fold higher MRT in the drought treatment. This pattern was accompanied by an increase in $\delta^{13}C$ in root WSOM. Beech is known to accumulate sugars as osmoprotectants, which increases osmotic pressure, maintains membrane integrity and stabilises proteins (Peuke *et al.* 2006). As a consequence, we can infer that new assimilates were utilised less intensively for respiration and other maintenance or growth-related processes.

In summary, we have to reject our first hypothesis: we neither found a significant reduction in the allocation of new assimilates belowground nor did we find a significant reduction in transport velocity. Thus, neither reduced phloem loading in source tissues nor impairment of phloem transport due to increased viscosity or failure to maintain phloem water status (cf. Sevanto 2014) occurs under the present experimental conditions. The inability of trees to maintain phloem transport functioning under water limitation is seen as one of the main reasons for tree mortality due to C starvation (Sala *et al.* 2010), but our results point to the fact that it is necessary to determine species-specific threshold values for drought intensity (Beier *et al.* 2012; Zang *et al.* 2014; Kayler *et al.* 2015) to characterise when a system is tipping from one state (not impaired or even increased assimilate allocation under moderate drought) to another (impaired and retarded assimilate transport under severe and potentially longer drought).

The effect of drought plus interspecific competition on the allocation of new assimilates

We initially hypothesised that the allocation of new assimilates to belowground tissues in mixed plantations would be strongly impaired in the drought-sensitive beech due to the combined effects of limited water availability and competition.

Under non-limited water supply (and drought), the interspecific competition treatment led to a clear increase in biomass of all compartments (leaves, stems, roots) in maple and to a decrease in beech compared to the monospecific controls. While the average assimilation rate of maple was not different between mono- and mixed cultures under well-watered control conditions, beech assimilation was negatively affected in the mixed culture. These findings point to a competitive advantage of maple over beech under non-limited water conditions, which is in agreement with the results of Simon *et al.* (2010), who assessed the nitrogen relation of both species. These authors found that beech was impaired in its nitrogen uptake when growing together with sycamore maple, but maple in turn benefitted from the interaction and increased soil nitrogen uptake. Nitrogen is the major growth-limiting nutrient in terrestrial ecosystems and C and nitrogen balance are closely coupled (Gessler *et al.* 2004). Thus, comparable patterns for nitrogen root uptake and C assimilation under competition are to be expected.

When water supply was not limited, maximum leaf $\delta^{13}C$ WSOM values for both beech and maple were smaller in mixed cultures compared to monocultures. The $\delta^{13}C$ patterns could not be explained by differences in leaf biomass between the competition treatments, since the absolute ^{13}C incorporation in leaf WSOM was also smaller: with ten individuals per tub, the total amount of ^{13}C recovered in WSOM 2 h after labelling began was 3.07 mg ^{13}C for maple monocultures, 4.14 mg ^{13}C for beech monocultures, but only 1.15 mg ^{13}C in the mixed cultures (five maple and five beech individuals). We might assume that the interspecific competition treatments incorporated relatively more of the new ^{13}C-labelled assimilates into non-water soluble storage compounds in leaves, stems and roots.

In general, there was no clear drought effect on $\delta^{13}C$ in WSOM in leaves, phloem and xylem, and no difference in the drought response of mixed cultures compared to the monocultures. In maple, the main difference between drought and control might be seen in a stronger initial ^{13}C signal of control trees in the phloem. This finding, along with the increase in MRT of leaf WSOM under drought in mixed cultures, might point to a drought-induced slowing down of phloem loading and transport for maple, as previously observed for other species (Ruehr *et al.* 2009; Barthel *et al.* 2011).

In contrast to maple, MRT of beech leaf WSOM decreased during drought and, consistent with the monoculture treatment $\delta^{13}C$ in root WSOM, was higher in the drought compared to the control treatment. Moreover, drought led to a 3.4-fold increase in the maximum ^{13}C excess in roots. These results imply that drought plus competition had no negative affect on belowground transport of new assimilates in beech. Our findings on the effects of drought and competition on C transport are partially in contrast to observations on the interaction between drought and competition on nitrogen uptake and allocation in beech (Fotelli *et al.* 2002). In this previous

study, drought not only increased the negative effects of the strong competitor *Rubus fruticosus* on beech ^{15}N uptake by the roots, but additionally led to impaired nitrogen allocation belowground. We, however, need to consider that nitrogen allocation might be subjected to different environmental and internal controls than C transport within the plant, and also that the moderate drought intensity applied in our study was not comparable to this other study. Fotelli *et al.* (2002) applied their drought treatment longer (50 days) and decreased soil water potential to values as low as -1.2 MPa, clearly below the root water uptake thresholds of soil moisture tension. The stronger severity of their drought resulted in clearly reduced root biomass (Fotelli *et al.* 2001), which is in contrast to our findings.

With respect to C, in both species of our study a larger proportion of new assimilates was transported belowground under drought plus interspecific competition compared to (i) non-limited water supply plus interspecific competition; and (ii) drought plus monospecific competition (*i.e.* monocultures). This points to the fact that under such conditions, both species invest significant resources belowground in order to more efficiently exploit water and potentially also nutrient resources, as well as to keep roots functional under osmotic stress.

We can state that maple was a superior competitor over beech under non-limited water conditions, as indicated by the changes in biomass of both species in the interspecific competition treatments, as well as the clearly reduced total ^{13}C label incorporation in WSOM of beech. However, as a consequence of our results, we also have to reject our second hypothesis: beech was still able to efficiently allocate new assimilates belowground under moderate drought and interspecific competition, and even increased the relative amount in the mixed cultures compared to the monocultures. This might be seen as a strategy to maintain the root osmotic potential and to prioritise root functioning. Our short-term study, however, precludes us from predicting how moderate drought and intraspecific competition would affect C allocation belowground and thus root growth and functioning in beech over the longer term.

In summary, our results clearly show that physiological responses of trees do not necessarily linearly follow the intensity of environmental drivers. In our case, a primary assumption was that the moderate drought would have comparable, although potentially weaker, effects (Blessing *et al.* 2015) compared to the stronger drought intensities applied in previous research (Ruehr *et al.* 2009). However, we neither found a clear reduction of the allocation of new assimilates from the leaves to sink tissues, nor did we detect strong indications for reduced transport velocity. On the contrary, beech allocated relatively more new assimilates belowground under moderate drought compared to non-limited water supply, and this pattern was even more pronounced under interspecific competition.

The drought-induced inhibition of C transport within plants is crucial for understanding the mechanisms of C starvation, which is one of the most intensively discussed factors for tree mortality under water limitation (McDowell *et al.* 2008; Sala *et al.* 2010). Recent studies (Beier *et al.* 2012; Kayler *et al.* 2015) have called for new experiments with multiple levels of a given environmental driver to characterise trajectories and thresholds in species-specific responses. Our results strongly support this suggestion as it shows, together with the previous work of Ruehr *et al.* (2009) and others, that depending on the level of drought, allocation of recent assimilates belowground can be either stimulated or reduced.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Schematic experimental set up of beech and maple in mixed treatment.

Figure S2. Whole plant transpiration rate (E_{plant}) of beech and maple in the drought and competition treatments.

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