Substrate influences ecophysiological performance of tree seedlings

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Unfavourable soil conditions frequently limit tree regeneration in mountain forests on calcareous bedrock. Rocky, shallow organic soils on dolomite pose a particular problem for tree regeneration due to commonly restricted water and nutrient supplies. Moreover, an often dense layer of understorey vegetation competes for the limited resources available. Hence, an array of interacting factors impairs tree seedlings’ performance on dolomite, but there is little information on the ecophysiological mechanisms. We studied the effects of substrate, competing vegetation and foliar nutrient concentrations on the photosynthetic rate (A), stomatal conductance (gs) and leaf water potentials (ψ) of sycamore (Acer pseudoplatanus L.), beech (Fagus sylvatica L.), spruce [Picea abies (L.) Karst.] and larch (Larix decidua Mill.) under controlled (well-watered/drought-stressed) conditions and under prevailing field conditions. While A and gs of well-watered spruce in the pot experiment were reduced by the mineral substrate, the organic dolomite substrate with dense competing vegetation reduced gs and ψ of sycamore, spruce and larch under drought-stressed conditions in the field. For sycamore and spruce, A and gs were strongly correlated with foliar nitrogen (N) and potassium (K) concentrations in the pot experiment. In contrast, soil water primarily affected beech and larch. Finally, dense competing vegetation negatively affected A and gs of spruce and A of larch on dolomite. Our results highlight the critical role of N, K and water availability for tree seedlings in shallow soils on calcareous bedrock. On these sites, natural tree regeneration is at particular risk from episodic drought, a likely consequence of climate change.

Keywords: competition, drought, foliar nutrient concentrations, Folic Histosol, gas exchange, multiple stresses, Northern Calcareous Alps, soil types.

Introduction

Tree regeneration frequently fails under unfavourable soil conditions in mountain forests (Brang 1998, Hanssen 2003, Diaci et al. 2005, Pröll et al. 2015). Shallow soils on rocky terrain such as Folic Histosols and Rendzic Leptosols commonly exhibit low soil water storage capacities, and soil matrix potentials are frequently low even in areas with high precipitation (Dale et al. 2001, IUSS Working Group WRB 2006, Stöhr 2007). Rocky shallow soils, which sometimes consist only of a thin organic or topsoil layer, are characterized by high drainage rates and a low water holding capacity (Hanssen 2003, Novák and Kňava 2012). Disturbances in mountain forests result in increased solar radiation, temperature, vapour pressure deficit and wind at the ground surface (Aussenac 2000, Prescott 2002, von Arx et al. 2012). As a result, the water demand of plants can exceed the water supply from the soil, increasing drought stress (Chaves et al. 2002, Larcher 2003). The phenomenon of high surface temperatures, usually coupled with drought in the upper soil horizons (Brang 1998, Hanssen 2003), is even more pronounced on cleared (O’Connell et al. 2004, Roberts et al. 2005), south-exposed sites with dark organic layers (Brang 1998, Diaci et al. 2005).

Frequently, low soil water content is coupled with reduced nutrient supply due to decreased mineralization, limited water
uptake and/or altered carbon (C)-partitioning with reduced fine root growth that further diminishes the active uptake of nutrients (Barber 1962, Chapin 1980, 1991, Oren and Sheriff 1995). The low nutrient availability is particularly pronounced on inherently nutrient poor soils such as organic soils on dolomitic bedrock, which frequently induce severe nutrient deficiencies, particularly of nitrogen (N) and phosphorus (P) (Glatzel et al. 1990, Baier 2004, Ewald 2005). Furthermore, a surplus of calcium (Ca) and magnesium (Mg) reduces the availability of other nutrients such as potassium (K), iron (Fe) and manganese (Mn) on dolomite due to ion-antagonism (Marschner 1995). In turn, nutrient deficiencies negatively influence the water status of trees (Sheriff et al. 1986, Oren and Sheriff 1995). In particular, low K supply reduces plant hydraulic conductance, which further reduces leaf water potential and stomatal conductance (Oddo et al. 2014). Hence, plants on nutrient poor soils such as organic soils on dolomite are particularly vulnerable to drought stress.

In addition to abiotic environmental factors, competition determines resource availability for trees (Hulme 1996, Nakashizuka 2001, Beckage and Clark 2003, Ladd and Facelli 2005). The pioneer vegetation spreading after disturbance can not only compete with tree seedlings (Lieffers et al. 1993, Diaci et al. 2005, Bloor et al. 2008) but also facilitate their establishment (Bertness and Callaway 1994, Diwold et al. 2010). The latter is frequently important for stress-intolerant species under stressful conditions (Callaway 1995, Choler et al. 2001, Liancourt et al. 2005). For example, dense understorey vegetation may favour regeneration of drought-sensitive species by producing shade and enhancing relative humidity in the canopy layer on open, sun-exposed sites. In contrast, dense understorey vegetation was shown to strongly impair the establishment, growth and physiological performance of tree seedlings due to intensified competition for limited soil resources (e.g., Löf 2000, Fotelli et al. 2001, Coll et al. 2004). Considering that shallow soils typically result in limited rooting space (Larcher 2003), we hypothesize that competitive effects may supersede facilitation effects. One of the main aims of this study is, thus, to investigate the effects of different substrates on the ecophysiology of tree seedlings under drought on shallow soils on calcareous bedrock and the effect of competing vegetation.

Despite the potentially high vulnerability of forests in the Calcareous Alps to drought stress in the future (Seidl et al. 2011, Thom et al. 2013), studies on the ecophysiology of tree seedlings growing on shallow soils typical of these mountain areas are scarce (cf. Sterck et al. 2008, Zweifel et al. 2009). The frequent failure of natural tree regeneration on disturbed mountain forest sites underlines the importance of understanding interactions between stress factors that affect tree seedlings’ performance, such as unfavourable soil conditions, nutrient limitations and drought. Due to the heterogeneity of substrates in the field, which represents a major challenge for controlled experiments, we initialized a pot experiment with seedlings of sycamore (Acer pseudoplatanus L.), beech (Fagus sylvatica L.), spruce (Picea abies (L.) Karst.) and larch (Larix decidua Mill.) on different substrates. The chosen species are typical of these mountain areas and exhibit a variety of ecological characteristics, including different tolerance to drought (Kubiske et al. 1996, Niinemets and Valladares 2006). Larch is a pioneer and light-demanding species, while spruce, sycamore and beech are moderately shade-tolerant to shade-tolerant species (Ellenberg 1996). Spruce, with a shallow rooting system, is considered to be more vulnerable to soil water deficits than sycamore, beech and larch (Ellenberg 1996, Niinemets and Valladares 2006, Hartl-Meier et al. 2014, Zang et al. 2014). In the course of our study, we compared physiological measurements under induced drought in the pot experiment with field measurements during a summer drought and heat wave in 2013.

Our hypotheses were that: (i) rocky, organic soil on dolomite impairs the photosynthesis and growth of sycamore, beech, spruce and larch due to the poor nutritional status on the dolomite substrate; (ii) seedlings experience more drought stress when growing on a substrate of organic soil on dolomite when compared with other substrates due to the negative influence of nutrient deficiencies on the water status of trees; and (iii) dense vegetation does not facilitate seedling growth but negatively affects different tree species by competing for limited resources and rooting space on shallow organic soils.

**Materials and methods**

**Pot experiment**

One-year-old, bare root, nursery-grown tree seedlings of sycamore (A. pseudoplatanus), beech (F. sylvatica), spruce (P. abies) and larch (L. decidua) were planted in April 2011. The seedlings’ initial height was between 20.4 ± 0.5 cm (spruce), 24.8 ± 0.8 cm (larch), 26.8 ± 1.6 cm (sycamore) and 28.2 ± 0.8 cm (beech) and similar for each species and substrate (mean height differences <3 cm). We planted 20 replicates of each species into three different substrates, each with and without competing vegetation. Hence, in total, we kept 480 individuals in 2.0-l pots (0.2 m deep) under open-air conditions at the experimental garden Knödelhütte in Vienna (48°13’8”N, 16°14’29”E). Potting substrates were collected from forest gaps in the Höllengebirge mountain range (47°47’19”N, 13°38’21”E) in the Northern Calcareous Alps, Austria.

The three different soil types were Folic Histosol on Wetterstein limestone (organic soil on limestone—OL), an intermediary between Rendzic Leptosol and Chromic Cambisol colluvium (mineral soil—MS) and Folic Histosol on dolomite (organic soil on dolomite—OD), according to IUSS Working Group WRB (2006). Humus forms were mull in the MS and moder in the organic soils, and humus horizons were OL, OF, OH and A, according to Zanella et al. (2011). Whereas MS consisted of...
one A–B horizon 0.18 m deep, collected as a disturbed soil sample (topsoil 0–0.2 m), OL was composed of three different organic layers in different proportions: two parts (0.07 m in depth) of undisturbed OL, two parts (0.07 m) of disturbed OF and one part (0.04 m) of disturbed OH. The C-horizon of the latter substrate comprised one calcareous stone of similar size in all pots. Likewise, OD consisted of three parts of undisturbed OL/OF (0.09 m in depth), one part of disturbed OH/C (0.03 m) and two parts (0.06 m) of fine-grained disturbed C-material (dolomite). The bottom of each pot was lined with a double layer (0.02 m in depth) of lightweight expanded clay aggregate and a thin fleece layer in order to improve water drainage and prevent the soil from spilling out. Each tree was provided the same amount of soil per substrate.

Half of the individuals planted in organic substrates were grown with established competing vegetation from the sampling site (C+) and half of them without (C–). Competing vegetation was trimmed if it began to develop in the control treatment (C–). For the disturbed mineral substrate, we expected the competing vegetation to establish from seedbanks during the first year of the experiment. However, in contrast to the organic dolomite substrate, which had extremely dense mats of Carex alba, competing vegetation on both the organic limestone and mineral substrate was negligible throughout the experiment, and hence, the factor was omitted for subsequent analyses of the substrate effects and the effect of competing vegetation could only be tested for OD.

To schedule irrigation, 0.15-m-long tensiometers (Jet Fill Tensiometer, Soilmoisture Equipment Corp., Santa Barbara, CA, USA) were installed in two pots per treatment. Tree seedlings in the pots with tensiometers were selected to be of similar height. During the 2011 growing season and pre-drought in 2012, tree seedlings were well-watered manually ensuring that the soil water potentials never dropped below −0.04 MPa.

### Tree morphology and competing vegetation of the pot experiment

The height of the 1-year-old tree seedlings was first recorded in April 2011 and again in August 2012. We also recorded the length of the terminal shoot increment in August 2011 and 2012. For estimating the total leaf area (LA), we harvested the total leaf biomass of each tree seedling in September 2012. Fresh leaves were scanned (Epson Expression 10000XL) and the projected leaf area was computed with WinRhizo software (Regent Instruments Inc., Quebec, Canada). After scanning, the leaf material was dried at 85 °C until constant weight. For sycamore and beech individuals with <30 leaves, all leaves were scanned. For beech with >30 leaves, a subsample of 30 randomly selected leaves was scanned and weighed, and for spruce and larch, a subsample of 20 needles was scanned and weighed, and LA was calculated by scaling to the total leaf dry weight of each individual. In October 2012, we harvested the above-ground competing vegetation of each pot by cutting it at the ground surface and measured its dry weight.

### Chemical analyses of the pot experiment

Foliar nutrient concentrations of 1-year-old leaves from each individual of the drought experiment were measured in the laboratory of the Institute of Forest Ecology, University of Natural Resources and Life Sciences, Vienna. Nitrogen and C were analysed with LECO TruSpec CN Analyser (Leco Corp., St Joseph, MI, USA). Sulphur (S), P, K, Ca, Mg, Fe, Mn and zinc (Zn) were extracted by microwave digestion in HNO3/HClO4 and measured by inductively coupled plasma optical emission spectrometer analysis (Optima 8300, Perkin Elmer, USA). Nine individuals of larch were excluded from analyses due to insufficient sample material. Nutrients were considered deficient if foliar concentrations were below critical values at which deficiency symptoms are typically observed on leaves in addition to growth reductions. We used critical values as reported for sycamore (Weber-Blaschke et al. 2008), beech and spruce (Mellert and Göttlein 2012) and larch (Göttlein et al. 2011).

### Physiological measurements during induced drought

The drought experiment with seedlings of sycamore and beech started on 4 August 2012 with 16 randomly selected individuals on each substrate (OL, MS and OD). At the beginning, we placed all pots into 0.05-m water-filled containers for several hours to assure water saturation from the bottom upwards in the pots. Then, half of the pots were saturated to field capacity with water each day, while the other half were kept without any water supply for 7 days until the first signs of wilting appeared. During the soil drying process, the plants were sheltered from rain in a plastic greenhouse with open walls.

Physiological measurements were conducted between 11:00 a.m. and 3:00 p.m. on 8–10 August. Photosynthesis (A), stomatal conductance (gₛ) and leaf water potential (ψ) measurements were carried out on four individuals each for both control and stressed trees, of each tree species and substrate (OL, MS and OD, each with and without competing vegetation). Since we only considered competing vegetation in the dolomite substrate, there were eight replicates for each of the organic limestone and the mineral substrate and four replicates for each of the organic dolomite substrates with (OD C+) and without (OD C–) competing vegetation. A and gₛ were recorded for the uppermost, fully expanded, sun-exposed leaf with a LI 6400 XT portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) using the broad-leaf chamber until steady state was reached. The leaf chamber was controlled to 1500 μmol m⁻² s⁻¹ photosynthetically active radiation with an artificial light source (6400-02B Red/Blue LED Light source), 400 p.p.m. CO₂, 30 °C leaf temperature and 40–50% relative humidity. After gas exchange measurements, the leaves were protected from excessive water loss by covering with a plastic bag, then cleanly cut and immediately placed inside
a sealed pressure chamber (Scholander et al. 1965) for leaf water potential measurements.

The drought experiment on seedlings of spruce and larch started on 11 August 2012, also with 16 randomly selected individuals of each substrate (OL, MS and OD) and followed the same measurement design as described above. For conifers, gas exchange measurements were conducted on the uppermost fully developed lateral branch with a LI 6400 XT conifer chamber (6400-2LL, LI-COR Inc.) on 15−17 August. Immediately after gas exchange and leaf water potential measurements, individual twigs were put in folded bags of aluminium and kept cool for leaf area measurements that enabled calculations of gas exchange parameters per unit leaf area. Needles were scanned with 2400 d.p.i. (Epson Expression 10000XL), and the leaf area was computed with WinRhizo software (Regent Instruments Inc.).

**Field measurements**

Plants were measured on two south-exposed disturbed sites at ∼950 m above sea level (a.s.l.) in the Höllengebirge mountain range of the Northern Calcareous Alps, Austria. One site is situated on Wetterstein limestone (47°48′10″N, 13°35′16″E) and the other on dolomite (47°47′35″N, 13°32′54″E). Measurements for OL and MS were conducted on the limestone site and for OD on the dolomite site. The soils comprise a mosaic of a Folic Histosol, Rendzic Leptosol and Chromic Cambisol according to the World reference base system (IUSS Working Group WRB 2006). The disturbed site on limestone originated from the catastrophic windstorm ‘Kyrill’ in January 2007. On that site, competing vegetation was still patchily dispersed in the measurement year 2013. The disturbed site on dolomite was affected by fire in 1906, numerous avalanches and historically intensive forest grazing. Current heavy browsing by ungulates and dense competing vegetation, mainly graminoids, impede tree establishment. Due to poor natural regeneration, post-disturbance reforestation was conducted on both sites.

The potential natural vegetation on the limestone site corresponds to a mixed beech forest with sycamore, spruce, fir (Abies alba Mill.) and ash (Fraxinus excelsior L.), while on the dolomite site, this forest community would transition towards an open pine forest (Pinus sylvestris L.) with spruce and larch. According to long-term records (1961−90) of the closest meteorological station (ZAMG - Central Institution for Meteorology and Geodynamics) and interpolation to 1000 m a.s.l. using functions of Harlfinger and Knees (1999), mean annual air temperature and precipitation are 5.8 °C and 1384 mm, respectively. Snow falls from October until April.

Physiological measurements during summer drought and the heat wave of 2013

For the gas exchange measurements, we selected similarly sized seedlings of the same species on the same substrates as used for the pot experiment. Similar to the pot experiment, competing vegetation was sparse on OL and MS and very abundant on OD. In contrast to the pot experiment, competing vegetation was not controlled in the field. Measurements were conducted on 16, 17, 22 and 24 July, and 1 and 2 August, during the summer drought and heat wave in 2013 (Figure 1). We measured 9 (OD) to 13 (OL, MS) individuals each of sycamore and beech and 5 (OD) to 10 (OL, MS) individuals each of spruce and larch per substrate. Gas exchange measurements were conducted with the same method as for the pot experiment. After gas exchange measurements, the measured leaves/twigs were cleanly cut, immediately put in folded bags of aluminium foil and kept cool, as proposed by Karlic and Richter (1979), for leaf water potential measurements at ∼4:00−5:30 p.m.

**Soil water content in the experimental garden and in the field**

For estimating the soil water status of each individual during the physiological measurements in the experimental garden and in the field, we measured the volumetric water content of soil with time-domain reflectometry (Fieldscout, TDR 100/200, Soil Moisture Meter, Spectrum Technologies, Inc., Plainfield, IL, USA), using 0.07-m-long rods. Measurements were each taken three times within 0.1 m of the stem. To obtain maximum accuracy, we developed calibration curves for the TDR instrument in each of the horizons (OL, OF, OH, A and B) of Rendzic Leptosols and Chromic Cambisols. In addition, we determined the relationship between volumetric water contents and soil matrix potentials (pF curves) for organic layers and mineral soil horizons using the Hyprop instrument (UMS, Munich, Germany). Additionally, soil matrix potentials were continuously recorded at a soil depth of 0.07 m with gypsum blocks and a micro data logger (MPS 1 sensors, EMS, Brno, Czech Republic) up to a range of −1.1 MPa.
in permanent plots on the disturbed site on limestone. Hence, we received continuous records of soil matrix potentials on the organic soil on limestone with (OL C+) and without (OL C−) competing vegetation and the MS (Figure 1). For OD, only discontinuous measurements were conducted.

**Statistical analysis**

Analysis of variance (ANOVA) tests were calculated for each tree species (sycamore, beech, spruce and larch) to determine the substrate effects (OL C−, MS C− and OD C− in the pot experiment; OL, MS and OD in the field) on ecophysiological parameters (A, gs and ψ). Response variables were log-transformed to obtain a normal distribution if necessary. Mean values of A, gs and ψ were compared with a Scheffé post hoc test at a significance level P < 0.05. Differences in A, gs and ψ between the control and drought-stressed treatment were tested by means of Welch’s t-tests for each substrate and species in the pot experiment. To test for differences in soil water potentials among substrates, we used ANOVA and Scheffé post hoc tests. Further statistical analyses were restricted to the pot experiment only.

We calculated a three-way ANOVA including drought (drought-stressed vs irrigated to field capacity), substrate (OL C−, MS C− and OD C−) and species (sycamore, beech, spruce and larch), and a two-way ANOVA to test the drought × substrate interaction separately for each species on A, gs and ψ. Effects of competing vegetation (yes/no) on various predictor variables (A, gs, ψ, ψsoil and foliar nutrient concentrations) were tested by means of Welch’s t-tests for each species on dolomite. ANOVA and Scheffé post hoc tests were used to test for effects of substrate on foliar nutrient concentrations (N, P and K) for each species. The same method was applied to test for effects of substrates on seedling height in 2012, shoot growth and LA (ANOVA and Scheffé post hoc tests between OL C−, MS C− and OD C− and Welch’s t-tests between OD C− and OD C+). We tested the correlations between A, gs and ψ of the four species and vapour pressure deficit between leaf and air (Vpd), volumetric water content of the soil (VWC), LA, foliar N and K concentrations and displayed the correlation coefficients in a correlation matrix. Further, we used multiple linear regressions to test for correlations between A, gs and ψ of the single species and various predictor variables (Vpd, VWC, LA, N and K). In the first step of the analysis, we used Vpd, VWC and LA as predictors (basic model—b0); in the second step, foliar N and K concentrations were each added (extended models—bN and bK). Finally, we performed a principal component analysis (PCA) to illustrate the effects and relevance of the different substrates on the parameters A, gs, Vpd, VWC, LA, foliar N, P, K, Ca, Mg, S, Fe, Mn and Zn. All statistical analyses were performed using R software 3.1.2 (R Core Team 2014).

**Results**

**Effects of substrate and drought in the pot experiment**

In the pot experiment, substrate had a significant effect on gs in beech and on the drought response of spruce (Table 1). Overall, during the drought experiment, a significant effect of substrate was only found for spruce (Figure 2). Among the well-watered trees, A was lower on MS C− than on OL C−, while gs was lower on MS C− than on OD C−. In the drought-stressed treatment, soil matrix potentials differed between the substrates (Figure 3a), but this did not necessarily affect ecophysiological parameters measured. Under drought conditions, no significant effect of substrate on physiological parameters could be determined.

| Table 1. Effects of drought treatment (drought stress vs control), substrate (OL C−, organic soil on limestone; MS C−, mineral soil and OD C−, organic soil on dolomite each without competing vegetation) and species (sycamore, beech, spruce and larch) as well as their interaction on photosynthetic rates (A), stomatal conductance (gs) and leaf water potential (ψ) in the pot experiment assessed by means of ANOVA (a). Only significant interactions are shown. The same tests were performed for the single species (b). ***P < 0.001, **P < 0.01, *P < 0.05, P < 0.1. |
|---|---|---|---|---|
| Species | Factors | A | gs | ψ |
| | F-value | P | F-value | P | F-value | P |
| (a) | | | | | | |
| All | Drought | 4.2 | * | 17.5 | *** | 14.9 | *** |
| | Substrate | 3.7 | * | 4.3 | * | 1.2 | |
| | Species | 31.4 | *** | 11.1 | *** | 58.7 | *** |
| | Drought × species | 1.1 | | 3.2 | * | 1.9 | |
| (b) | | | | | | |
| Sycamore | Drought | 0.7 | | 1.9 | | 0.0 | |
| | Substrate | 1.7 | | 1.9 | | 2.2 | |
| Beech | Drought | 3.9 | ~ | 10.2 | ** | 3.2 | ~ |
| | Substrate | 0.0 | | 4.6 | * | 1.0 | |
| Spruce | Drought | 0.1 | | 0.2 | | 0.9 | |
| | Substrate | 2.3 | | 3.0 | ~ | 1.0 | |
| | Drought × substrate | 3.8 | * | 2.2 | | 5.3 | |
| Larch | Drought | 2.4 | | 8.9 | ** | 11.6 | ** |
| | Substrate | 1.6 | | 1.6 | | 2.5 | ~ |
Among the four species, significant effects of drought were only recorded for beech and larch (Table 1, Figure 2). Spruce was affected by drought on MS only.

For all four species, tree morphological parameters strongly differed between the substrates (Table 2). Seedling height, shoot growth and the leaf area of sycamore, spruce and larch were lowest on MS C− compared with OL C− and OD C−. Similarly, beech showed highest values on OL C−, but in this case, compared with both MS C− and OD C−.

Effects of substrate and drought in the field compared with the pot experiment

Under field conditions, substrate had an effect on $g_s$ or $\psi$, but not on $A$ (Figure 4). All tree species except beech showed lower $g_s$ or $\psi$ on OD than on MS and OL. While sycamore responded with significantly lower $g_s$, larch responded with lower $\psi$ on OD than when grown on MS. Spruce also responded with lower $\psi$, however, with respect to both MS and OL. Opposing relationships to the soil matrix potentials were found: while $g_s$ or $\psi$ were highest, soil matrix potentials were lowest on MS (Figures 1 and 3b). In addition, sycamore, beech and larch showed lower $A$ on OD than on MS and OL, respectively, though in all cases, only with marginal significance ($P < 0.1$).

In general, all species except beech showed contrasting trends in water relations between the pot experiment and the
field measurements. In sycamore, $\psi$ in the pot experiment was substantially higher than in the field. In spruce and larch, gas exchange and $\psi$ showed little indication of stress in the pot experiment, but they strongly reduced gas exchange rates and had moderately low $\psi$ in the field.

**Effects of competing vegetation in the pot experiment**

Among the four species, spruce and larch were most sensitive to competing vegetation. While spruce showed lower values of $A$ and $g_s$ in both the well-watered and drought-stressed trees, larch showed lower values of $A$ in the well-watered trees on OD C− compared with OD C− (Figure 5). Interestingly, the effect of competition on ecophysiology was not reflected in an effect on soil water potentials (Table 3). However, in spruce, lower N and K concentrations were recorded on OD C+ compared with OD C−. Similar to physiology, seedling height, shoot growth and the LA of spruce and larch were lower on OD C+ than on OD C− (Table 2).

**Effects of environmental parameters and foliar nutrient concentrations in the pot experiment**

To test whether the poor nutrient supply affects photosynthesis and water relations, we analysed foliar nutrient concentrations for each species and substrate in the pot experiment (Figure 6). Concentrations of foliar P and K were below the critical values reported for all four species. For all species except larch, foliar N concentrations depended on substrate: N availability was sufficient on OL C−, while N deficiencies were detected on MS C− and OD C−. Similarly, foliar P was highest on OL C− and lowest on MS C− for spruce. For foliar K, similar patterns were found: higher concentrations of foliar K on OL C− than on the other substrates for larch.

Correlations between ecophysiological and environmental parameters and foliar nutrient concentrations revealed significant differences between the species. There was a strong positive correlation between foliar K concentrations and $A$ as well as $g_s$ ($r = 0.6–0.8$) and between $A$ and foliar N concentrations ($r = 0.5–0.6$) in sycamore and spruce (Figure 7). Soil water content (VWC) was closely linked to $g_s$ in beech and larch ($r = 0.5$). The Vpd showed a strong negative correlation with $A$ and $g_s$ ($r \geq 0.5$) for all tree species except spruce. Given the high correlations between N and K concentrations and $g_s$, significantly affected physiology and considerably increased the goodness of fit of the basic linear regression models (Table 4), in particular for sycamore ($R^2 = 0.7$). For beech and larch, the basic linear regression models revealed a strong relationship between Vpd and WVC and $g_s$ ($R^2 = 0.5$). For beech, leaf area also significantly affected $g_s$.

The PCA summarizes substrate effects on the respective species: the first two components (PC1 and PC2) accounted for 51.1% (sycamore), 49.9% (beech), 53.4% (spruce) and 44.9% (larch) of the variance (Figure 8). For all species, the first four principal components had eigenvalues >1. $A$ and $g_s$ were closely correlated in all four species. While $A$ and $g_s$ were associated with foliar K and N in sycamore, physiological parameters were not related to VWC and foliar P and S. In contrast, $A$ and $g_s$ of beech were mainly affected by WVC, while foliar nutrient concentrations were less important. Similar to sycamore, $A$ and $g_s$ of spruce were closely related not only to foliar K and N but also to S and P. Finally, $A$ and $g_s$ of larch mainly responded to VWC and VPD, while nutrients showed a minor influence. According to the PCA, OL was most distinct from other substrates, strongly differing in Mg and some other nutrients (Mn, N and S). With the exception of spruce, OD C− and OD C+ hardly differed among the different species.

**Discussion**

**Effects of substrate and drought in the pot experiment**

Under well-watered conditions, $A$ of spruce was highest on OL C− and lowest on MS C−. Similarly, the growth of all species was lowest on MS C− and poor nutrient supply was documented on OD C− and MS C−. Hence, our results only partially support hypothesis (I). Poor growth and vitality of trees on calcareous soils have been reported in previous studies and were mainly attributed to mineral nutrient deficiency due to soil chemistry (Baier 2004, Ewald 2005, Mellert and Ewald 2014).
Poor nutrient availability was even more pronounced on MS, as reported by Johnson et al. (1997) and Baier et al. (2006a). The reductions of $A$ and growth of tree seedlings on MS reported here are, therefore, not surprising. Moreover, low nutrient uptake efficiency of plants growing on MS may result from restricted fine root growth due to high soil bulk density (Tracy et al. 2012) and low abundance of ectomycorrhiza (Baier et al. 2006b).

Our study confirmed the sensitivity of photosynthetic capacity to mineral nutrient deficiencies for sycamore and spruce in accordance with earlier studies (Weikert et al. 1989, Kazda et al. 2004). In particular, N, which is closely associated with photosynthetic efficiency (Chapin et al. 1987, Reich et al. 1998, Binkley et al. 2004) and was deficient on MS C− and OD C−, may limit growth of tree seedlings on these substrates. However, K, which is strongly correlated with photosynthesis and growth (Basile et al. 2003, Tripler et al. 2006, Jin et al. 2011), was also limiting on all substrates. Hence, due to the close correlations between $A$ and foliar N and K, the low availability of N and K likely is the main limiting factor for photosynthesis and growth in sycamore and spruce.

Although correlations between $A$ and foliar N were high for both sycamore and spruce, N explained rather little of the variance in the corresponding models for spruce (Table 4). In fact, additional elements were correlated with the photosynthetic capacity of spruce, namely S and P (Figure 8). Several authors have addressed P limitation aside from N as a key factor for primary production in forest ecosystems (Flückiger and Braun 1995, Vitousek et al. 2010, de Vries and Posch 2011, Jonard et al. 2015). Likewise, Jonard et al. (2015) claimed that co-limitation of N and P and the scarcity of S have diminished the primary production of spruce in European forests in previous decades. In addition, soil water supply is decisive for the nutrient availability to trees (Peuke and Rennenberg 2004, Kreuzwieser and Gessler 2010). Hence, interacting effects of overall nutrient availability, frequency and duration of drought events in addition to CO$_2$ concentrations will primarily determine forest growth in the future (Körner 2006, 2013, Sigurdsson et al. 2013, Fernández-Martínez et al. 2014).

We found that while sycamore and spruce responded to nutrients, beech and larch were obviously affected by short-term drought. The high correlations between $g_s$ or $\psi$ and soil water
contents of the latter species strongly support the concept of anisohydric behaviour (Tardieu and Simonneau 1998) as reported in previous studies for beech and larch (Lévesque et al. 2013, Hartl-Meier et al. 2015). Although this strategy enables continuously high gas exchange rates during drought, the leaf water potential frequently drops down to a critical level where hydraulic failure sets in (McDowell et al. 2008). This strategy seems particularly risky for plants on shallow organic soils due to frequently restricted soil water supply. Here, an effective stomatal control that ensures relatively constant leaf water potentials would appear to be a good strategy against hydraulic failure. Sycamore and spruce follow this isohydric behaviour as indicated by the insensitivity of leaf water potentials to soil water contents during short-term drought. However, as McDowell et al. (2008) stated, seedlings with restricted rooting volumes are generally vulnerable to hydraulic failure, irrespective of their water regulation strategy. In this context, it is important to mention that the water stress period in the pot experiment was too short to study plant acclimation to drought.

**Effects of substrate and drought in the field**

Overall, under drought stress in the field, all species except beech were affected by substrates: on OD, sycamore had lower $g_s$ while spruce and larch had lower $\psi$. Thus, we cannot reject hypothesis (ii). Sycamore with $g_s < 50$ mmol m$^{-2}$ s$^{-1}$ and $\psi$ approximately $\sim -1.9$ MPa was severely drought stressed on dolomite. Considering

![Figure 5. Mean values and standard errors of midday photosynthetic rates (A), stomatal conductance ($g_s$) and leaf water potentials ($\psi$) of control (white) and stressed (black) seedlings (2-year-old) of sycamore, beech, spruce and larch on organic soil on dolomite (OD) without (C–) and with (C+) competing vegetation ($n = 4$). Different letters indicate significant differences (Welch’s t-test, $P < 0.05$) between the treatments C– and C+ within species and drought treatment (normal letters indicate differences within the control treatment and underlined letters within the stressed treatment). Asterisk indicates significant differences between the control and drought-stressed treatment of OD C– and OD C+: *$P < 0.05$.

![Table 3. Mean values ± standard errors of the biomass of competing vegetation (BM_compet in g dry weight; $n = 20$), soil matrix potentials ($\psi_{soil}$; $n = 4$) and foliar nutrient concentrations (N, P and K in mg g$^{-1}$ dry weight; $n = 8$) of sycamore, beech, spruce and larch on organic soil on dolomite (OD) without (C–) and with (C+) competing vegetation. Soil matrix potentials were measured within the top 0.1 m for drought-stressed seedlings. Bold font indicates significantly lower values for OD C+ compared with OD C–.](image-url)
that a xylem water potential of −1.5 to −2.2 MPa causes a 50% loss of hydraulic conductivity ($\psi_{50}$) (Lemoine et al. 2001, Tissier et al. 2004, Lens et al. 2011), the recorded values indicate high drought stress resulting in substantial cavitation in sycamore. As the conifers kept their stomata completely closed, leaf water potentials were around −2.0 MPa, above the $\psi_{50}$ of spruce and larch of approximately −3.0 MPa (Charra-Vaskou et al. 2012), so they avoided xylem dysfunction. Similarly, beech showed leaf water potentials of approximately −2.0 MPa, above the $\psi_{50}$ (approximately −2.5 to −3.0 MPa) reported by Cochard et al. (1999) and Wortemann et al. (2011).

The constant relatively high $g_s$ and $\psi$ of sycamore in the pot experiment strongly contrast with the effect of drought in the field—indicated by very low $\psi$. High transpiration rates and $\psi$ under well-watered conditions for sycamore were reported in previous studies and are attributed to high hydraulic conductance of stems and leaves (Lemoine et al. 2001, Nardini et al. 2012). Similarly, intermediate resistance to cavitation (Lens et al. 2011) and low capability of modulating the ionic concentration in the xylem sap (Nardini et al. 2012) were reported for sycamore. Hence, the sensitivity of sycamore to severe drought is not surprising. Moreover, our results indicate that spruce and larch were moderately stressed during summer drought, as was shown previously (Lebourgeois et al. 2010, Eilmann and Rigling 2012, Lévesque et al. 2013). Nevertheless, larch is considered to be more drought tolerant than spruce due to the capacity of osmoregulation (Anfodillo et al. 1998, Badalotti et al. 2000).

Overall, the field measurements indicate that sycamore, spruce and larch can better cope with drought stress on the MS with sparsely developed competing vegetation than on the inherently nutrient poor organic soil on dolomite with dense competing vegetation. This in spite of the very negative soil water potentials in the MS recorded within the top 0.1 m. It is possible that tree roots extended into deeper soil horizons to access water and nutrients when resources are scarce near the surface (Valentin et al. 1994, Puhe 2003), which would explain
the apparent contradiction between the higher values of eco-
physiological parameters at lower soil matrix potentials.

However, physiological parameters are not solely influenced
by soil water availability but also by nutrient supply and com-
petitive interactions (Nilsen and Orcutt 1996). These stresses
impair tree physiology, alone or through their interaction
(Niinemets 2010). For instance, low soil water contents and
dense competing vegetation can reduce nutrient availability
(e.g., Oren and Sherif 1995, Ladd and Facelli 2005). This
appears to be the case for sycamore, spruce and larch in the
field where the inherently nutrient poor organic soil on
dolomite with dense competing vegetation resulted in poor
performance.

Effects of competing vegetation on dolomite
in the pot experiment

The higher A in spruce and larch and the higher g_s in
spruce on dolomite without competing vegetation compared
with tree seedlings on the same substrate with dense competing
vegetation show the sensitivity of spruce and larch to competition
(Figure 5). Similarly, selected growth parameters and LA were
negatively affected by competing vegetation (Table 2). Hence,
we cannot reject hypothesis (iii). The sensitivity to competing
vegetation for larch and different species of spruce has been
documented elsewhere (Jobidon et al. 1998, Grossnickle 2000,
Dullinger et al. 2005) and supports our findings.

Similar to A and g_s, significantly lower foliar N and K concen-
trations were documented on dolomite with vs without compet-
ing vegetation in spruce. Given that A and g_s were closely related
to foliar N and K, we assume that the low nutrient supply limits A
and g_s in spruce on OD C+. In addition, under competition with
pioneer vegetation, tree seedlings can suffer from poor nutrient
supply, which was also seen in previous studies (Kronzucker
et al. 1997, Parker et al. 2012). Interestingly, the competition
for nutrients appears to be more important than for water, since
soil water supply was sufficient in the control treatment. In con-
trast, the effect of competing vegetation on soil water availability
was negligible under water stress in the pot experiment. Dense
competing vegetation may have little effect on soil water if the
effects on microclimate (Jobidon et al. 1998) reduce evapor-
atron from the soil surface (Burton and Bazzaz 1995). Together
with the results from our study, this suggests that competition for

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Table 4. Results of multiple linear regressions relating photosynthetic rates (A) and stomatal conductance (g_s) of sycamore, beech, spruce and larch in the pot experiment to various predictor variables. The basic models (b0) include vapour pressure deficits between leaf and air (Vpd_s), soil volumetric water content (VWC) and total leaf area (LA) as predictors; in the extended model bN, foliar N concentrations are also included, and in the extended model bK, foliar K concentrations are also included as predictors. ***P < 0.001, **P < 0.01, *P < 0.05. R^2 indicates goodness of fit of the respective models (b0, bN, bK).

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Vpd_s</th>
<th>VWC</th>
<th>LA</th>
<th>N</th>
<th>K</th>
<th>R^2</th>
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</tr>
<tr>
<td></td>
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<td>3.19***</td>
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<td></td>
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<td>1.37</td>
<td>12.7</td>
<td></td>
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<td></td>
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<td>0.15</td>
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<td></td>
<td>0.37</td>
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<tr>
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<td>0.09</td>
<td>0.04**</td>
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</tr>
<tr>
<td></td>
<td>bK</td>
<td>−0.04***</td>
<td>0.07*</td>
<td>−0.22</td>
<td></td>
<td>0.04***</td>
<td>0.63</td>
</tr>
<tr>
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<td>2.11</td>
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<tr>
<td></td>
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<td>−0.46*</td>
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<tr>
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<td>0.08*</td>
<td>−0.46*</td>
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<td>0.08*</td>
<td>0.29</td>
<td>0.05**</td>
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<td>−5.0</td>
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<tr>
<td></td>
<td>bL</td>
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<td>0.24***</td>
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<tr>
<td></td>
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<td></td>
<td>bK</td>
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<td>−0.56</td>
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nutrients rather than water is the limiting factor for some tree species growing on dolomite. Nevertheless, these results drastically contrast the findings of strong negative effects of competing vegetation on available soil water for species other than spruce (Davis et al. 1999, Coll et al. 2004). This was partly related to the fact that grass species can outcompete trees in water uptake (Coll et al. 2003, Taylor et al. 2014). Further investigations under severe drought are necessary to identify major causes for failure of spruce under dense competing vegetation on dolomite.

Conclusions

Photosynthesis and stomatal conductance of well-watered spruce are reduced on mineral soil, while shallow organic soil on dolomite with dense competing vegetation impairs the stomatal conductance or leaf water potential of drought-stressed sycamore, spruce and larch. Overall, sycamore and spruce are mainly affected by N and K supply, while soil water primarily affects beech and larch. Competing vegetation affects nutrient rather than water supply for tree seedlings. Our results show that tree

Figure 8. Biplots, representing PC1 and PC2 of PCA for (a) sycamore, (b) beech, (c) spruce and (d) larch. Symbols represent the different substrates of the pot experiment and ellipses the normal data ellipses for each substrate. Crosses indicate OL C− = organic soil on limestone without competing vegetation, filled circles MS C− = mineral soil without competing vegetation, filled triangles OD C− = organic soil on dolomite without competing vegetation and filled squares OD C+ = organic soil on dolomite with competing vegetation. Vectors indicate the parameters of the PCA (A, photosynthetic rate; gs, stomatal conductance; VWC, soil water content; Vpd, vapour pressure deficit between leaf and air; and leaf nutrient concentrations—N, P, K, Ca, Mg, S, Mn, Fe and Zn).
regeneration is particularly vulnerable on shallow calcareous soils in terms of limited nutrient availability and episodic droughts.

**Supplementary data**

Supplementary data for this article are available at Tree Physiology Online.

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**Conflict of interest**

None declared.

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