Responses of two closely related oak species, *Quercus robur* and *Q. petraea*, to excess manganese concentrations in the rooting medium

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Summary *Quercus robur* (L.) and *Q. petraea* (Matt.) Liebl. are European oak species that often grow in forest soils with high soluble manganese (Mn2+) concentrations. We tested the effects of Mn2+ at concentrations of 0.0024 mM (control), 0.24 mM (typical of acidic forest soils) and 1.2 mM (typical of forest soils under strongly reducing conditions) on the growth, tissue anatomy, foliar element concentrations, subcellular element distribution and gas exchange of solution-cultured seedlings. At the highest Mn2+ concentration, seedlings were grown with and without an elevated concentration (1.2 mM) of magnesium (Mg2+). At 0.24 mM Mn2+, foliar Mn concentrations were higher than observed in the field. Vacuoles of the leaf epidermis and mesophyll were the main sites of manganese accumulation. High nutrient solution Mn2+ concentration significantly lowered foliar iron (Fe) and Mg concentrations. Elevated Mg2+ concentration raised the foliar Mg concentrations to control values, but Fe concentrations and gas exchange remained depressed. In seedlings grown in the 1.2 mM Mn2+ treatment without elevated Mg2+, damage to the phloem of the petioles and a reduction in root mass were observed in both species. The effects on shoot and root growth were greatest in *Q. petraea*. Alleviation of manganese toxicity symptoms by Mg2+ in *Q. petraea* was less effective than in *Q. robur*. Our results suggest that the soil solution Mn2+ concentrations that occur in European oak forests are unlikely to affect the distribution and performance of *Q. robur* and *Q. petraea* in the field.

Keywords: biomass partitioning, EDX analysis, gas exchange, iron, magnesium, phloem damage, root growth, toxicity.

Introduction

Manganese (Mn) is an essential plant micronutrient that is part of the water-splitting system associated with photosystem II and, as Mn3+, activates many enzymes (Marschner 1995). In most plants, foliar Mn concentrations of more than 0.02 mg g⁻¹ dry mass (DM) are sufficient for normal growth, but they can vary by a factor of 60 or more among species, as well as among conspecific individuals, depending on site conditions (Bergmann 1993). Accordingly, critical concentration thresholds for Mn toxicity vary widely among trees. In a comparison of seedlings of four Australian tree species, the critical foliar Mn concentrations ranged from 0.27 mg gDM⁻¹ (Acacia holosericea A. Cunn. ex G. Don) to 7.23 mg gDM⁻¹ (Eucalyptus camaldulensis Dehnh.; Reichman et al. 2004). Apart from visible symptoms of toxicity (e.g., Horst 1988), uptake of excess Mn2+ can result in growth reduction and impairment of chlorophyll synthesis (Bergmann 1993), lowered foliar concentrations of iron (Fe) and magnesium (Mg) (Marschner 1995) and decreased gas exchange (Kitao et al. 1997, St. Clair and Lynch 2005). Excess Mn2+ is known to inhibit Mg2+ uptake through ion competition at absorption sites on the root surface (Wang et al. 1992, Alam et al. 2003). Within the cell, excess Mn2+ can displace Mg2+ from ATP, thereby disturbing the energy-transmitting system, and can depress net photosynthesis through inhibition of the ribulose-1,5-bisphosphate carboxylase reaction (Marschner 1995, Lidon et al. 2004, Manter et al. 2005). In leaves, cell walls are the main sites of accumulation of excess Mn (Dučić et al. 2006). Manganese-induced Mg deficiency is likely the cause of manganese-induced phloem damage observed in Acer saccharum Marshall (McQuattie and Schier 2000) and Picea abies (L.) H. Karst. (Keil et al. 1986, Fink 1991, Puech and Mehne-Jakobs 1997).

Manganese is taken up from the soil solution as Mn2+ (Clarkson 1988), the abundance of which depends on soil oxidation potential and pH: Mn2+ is prevalent at oxidation potentials up to 0.6 V at pH 6 (Dixon and Skinner 1992), and its concentration increases with decreasing pH and oxidation potential (Norvell 1988). Thus, soil acidification and waterlogging result in increased Mn2+ availability and uptake. In Europe, Mn toxicity has been observed in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and has been attributed to soils acidified by acidic deposition (Schöne 1987, Schöne 1992, Kaus and Wild 1998). During the past several decades, declining health has been recorded throughout large parts of Europe in the closely related oak species *Quercus petraea* (Matt.) Liebl. and *Q. robur* L. (Delatour 1983, Schlag 1994, Hartmann 1996). However, it is unknown to what extent this decline results from increased Mn2+ availability due to soil acidification. In 17 mature oak stands monitored in northern Germany, Mn was the only nutrient in the soil solution that significantly cor-
related with its concentration in leaves (Büttner 1997). This relationship prompted the present study to assess the role of Mn²⁺ availability on the development of injury in *Q. petraea* and *Q. robur*.

*Quercus petraea* and *Q. robur* differ in their ecological responses to site conditions: *Q. robur* is less susceptible to high water tables and waterlogging than *Q. petraea* (Lévy et al. 1986, Wagner and Dreyer 1997, Schmull and Thomas 2000). We hypothesized that *Q. robur* would be less susceptible to increased Mn²⁺ concentrations in the rooting medium. In oak stands in north-central Europe that experience temporary waterlogging, a combination of low pH and high Mn²⁺ concentration can occur in the topsoil (e.g., Thomas and Büttner 1998) and may be important in determining the success of seedling establishment. We, therefore, studied oak seedlings in solution culture, with well-defined nutrient ratios and controlled pH. The nutrient ratios were similar to those of soil solutions obtained from oak stands in northern Germany (Thomas and Büttner 1998). In the high Mn treatments, Mn²⁺ was supplied at concentrations typical of the upper range of concentrations in acidic or reduced forest soils. In another treatment, Mg²⁺ was supplied at the same molarity as the high Mn²⁺ concentration, to determine whether it could counteract Mn²⁺ toxicity (Marschner 1995).

We tested the following hypotheses: (1) excess Mn²⁺ in the nutrient solution results in decreased foliar concentrations of Fe and Mg; (2) in leaves, excess Mn is mainly deposited in the cell walls; (3) by damaging the phloem of source leaves, high Mn²⁺ affects biomass partitioning; (4) biomass production of *Q. robur* is less susceptible to excess Mn²⁺ than that of *Q. petraea*; and (5) Mg²⁺ alleviates Mn²⁺ toxicity, with *Q. robur* benefiting more than *Q. petraea*.

### Materials and methods

**Plant cultivation, biomass production and foliar element concentrations**

Acorns of sessile oak (*Quercus petraea*) were obtained from the Forest Seed Centre in Oerrel (Lower Saxony), and those of pedunculate oak (*Q. robur*) from a tree nursery (Rahite; Wietze, Lower Saxony). The acorns were kept in moist sand until they germinated in mid-April. After rinsing the roots with tap water, seedlings were placed in 1.5-l pots filled with tap water and, after 4–5 days, transferred to pots filled with half-strength nutrient solution for 1 week, followed by full-strength nutrient solution: 2.96 mM nitrogen (N); 1.96 mM NO₃⁻ + 1 mM NH₄⁺; 0.1 mM phosphorus (P); 0.86–3.02 mM sulfur (S), depending on the Mn²⁺ concentration; 0.24 mM calcium (Ca); 0.24 mM Mg; 0.96 mM potassium (K); 0.048 mM Fe; 4.5 μM boron; 2 μM zinc; and 1.2 μM molybdenum. The nutrient ratios were similar to those found in solutions obtained from the topsoil of an oak stand in northern Germany (Table 1). Twelve weeks after the start of the experiment (at the beginning of August), the plants were transferred to 12-l pots (one plant per pot), containing the same nutrient solution.

Within species, seedlings were assigned to one of four treatments differing in Mn²⁺ and Mg²⁺ concentrations (Table 1). Control seedlings received Mn²⁺ and Mg²⁺ at concentrations of 0.0024 and 0.24 mM, respectively. Other treatment solutions contained Mn²⁺ at concentrations of 0.24 (MnA treatment) and 1.2 mM (MnR treatment), typical of the soil solution of acidic and reduced forest soils, respectively. In a fourth treatment (MnMg), both Mn²⁺ and Mg²⁺ were supplied at a concentration of 1.2 mM, as sulfates. The pH of the nutrient solutions was adjusted to 4.5 every 3–4 days with potassium hydroxide or sulfuric acid. Immediately before adjustment, the pH was between 3.2 and 4.6, depending on the growth rate of the plants and the pot size. The nutrient solutions were constantly aerated, and replaced at weekly intervals. For the duration of the solution culture (22 weeks from the beginning of May to the beginning of October), plants were kept in a greenhouse (51°33′N, 9°56′E, 150 m a.s.l.) in a 14-h photoperiod (0600 to 2000 h), day/night temperature of 20/15 °C and 60 ± 15% relative humidity. During the day, additional light (135 µmol m⁻² s⁻¹ photosynthetic photon flux (PPF); HQIE/DV 400, Osram, Munich, Germany) was supplied at plant height. Plant positions in the greenhouse were randomly changed every week. Ten plants per species were used for each treatment.

At harvest in mid-October, plant height and root length were measured, and the plants subdivided into the shoot axis (including twigs), first-flush leaves (developed at the beginning of May), second-flush leaves (generated in seven to ten plants per species and treatment at the end of June), third-flush leaves (developed up to five plants per species and treatment in August), fine roots (diameter ≤ 2 mm) and coarse roots (diameter > 2 mm). The plant material was oven-dried to constant mass at 105 °C. Dried leaves were pulverized and digested with nitric acid (65%). Foliar concentrations of Ca, Fe, K, Mg and Mn in the digests were analyzed by atomic absorption spectrometry (SpectrAA-30, Varian, Palo Alto, CA).

**Gas exchange**

Eight and 16 weeks after the start of the experiment, stomatal conductance to water vapor (gₛ) of two or three first- and second-flush leaves per seedling were measured with a porometer (LI-1600, Li-Cor, Lincoln, NE) under ambient conditions in the greenhouse. At the end of the experiment, additional measurements of net photosynthesis were conducted on one first-flush leaf per seedling of two seedlings in each of the control, MnR and MnMg treatments. Measurement were made with a minicuvette system (CMS 400, Walz, Effeltrich, Germany) at 20 °C and 60% humidity in five steps per leaf: (1) 250 ppm CO₂, PPF 360 µmol m⁻² s⁻¹ (light saturation in oak seedlings grown under greenhouse conditions: Thomas 1991); (2) 370 ppm CO₂, PPF 360 µmol m⁻² s⁻¹; (3) 1000 ppm CO₂, PPF 360 µmol m⁻² s⁻¹; (4) 1000 ppm CO₂, PPF 54 µmol m⁻² s⁻¹; and (5) 1000 ppm CO₂, PPF 0 µmol m⁻² s⁻¹. Net photosynthesis at light saturation and ambient CO₂ concentration (Aₚₙₓ), photosynthetic capacity at saturating light and elevated CO₂ concentration (Aₚₙₓ), instantaneous water-use efficiency (WUE; Δₚₙₓ per unit transpiration) and maximum carboxylation rate (Vₚₚₚₓ) were determined according to Farquhar et al. (1980).
After completion of the photosynthetic measurements, chlorophyll concentrations were determined in the remaining first-flush leaves of seedlings in the control, MnR and MnMg treatments (five to nine leaves per treatment for *Q. robur*, two to three leaves per treatment for *Q. petraea*). Two leaf discs per leaf (0.38 cm² per disc) were punched out with a cork borer. After addition of 2 ml of N,N-dimethylformamide (DMF), the samples were stored in a refrigerator for 24 h. Absorption was measured (UV 160, Shimadzu, Kyoto, Japan) at 664, 647, 625 and 603 nm against pure DMF. Chlorophyll concentrations were calculated according to Moran (1982) and were related to leaf area.

**Microscopic and EDX analyses**

At harvest, two first-flush leaves per plant (three plants per treatment) were randomly sampled from the control and MnR treatments. Fine roots were obtained from two plants in both the control and MnR treatments. From the petiole and blade of each harvested leaf, and from the rhizodermal zone of the fine roots, up to five cross sections (1–2 mm wide) were cut with a razor blade and quick-frozen in a 2:1 (v/v) mix of propane and isopentane cooled to −196 °C in liquid nitrogen. The samples were lyophilized and stored over silica gel.

The lyophilized samples were infiltrated with diethyl ether under vacuum and embedded in 1:1 (v/v) styrene:butyl methacrylate with 2 g of dibenzoyl peroxide, which was polymerized at 60 °C for 20 h, then at 35 °C for 10 days. One-µm-thick sections were cut with an ultramicrotome (Ultracut 2, Reichert and Jung, Vienna, Austria).

For light microscopy, sections were transferred to microscope slides, straightened with chloroform vapor and fixed at 60 °C. Petiole sections were stained with gentian violet (0.1%), and foliar transverse sections and root cross sections stained with toluidine blue (0.1% in 0.2 M phosphate buffer, pH 7). Stained sections were analyzed with a light microscope (M35, Zeiss, Oberkochen, Germany). Sections were photographed through a gray and a blue filter (Kodak Ektachrome 100 HC daylight filmstrip).

For transmission electron microscopy (TEM), transverse leaf sections were mounted on 100-mesh hexagonal grids (Plano, Wetzlar, Germany), sputtered with carbon under vacuum and stored over silica gel until imaged with a transmission electron microscope (EM 420, Philips, Amsterdam, The Netherlands) coupled with an energy dispersive X-ray (EDX) analysis system (EDAX 9100, EDAX, Mahwah, NJ). The accelerating voltage was 120 kV, and the counting time was 40 live seconds. Concentrations of Mn and P were measured in the vacuoles of leaf epidermis, palisade parenchyma and spongy mesophyll cells, or in the cell lumina of phloem cells, and in the cell walls of these tissues, then corrected against background measurements of 1:1 (v/v) styrene:butyl methacrylate. For correction of the different detection sensitivities of the individual elements, measured peak intensities were multiplied by element-specific factors according to Cliff and Lorimer (1975). From the corrected peak intensities, element concentrations were calculated according to Hall (1971) and Fritz and Jentschke (1994). Photographs were taken from selected tissue sections with black-and-white film (Ilford, Mobberley, U.K.).

**Statistical analyses**

Means ± 1 standard error (SE) are presented, unless otherwise indicated. Most of the datasets were not normally distributed, according to the Shapiro and Wilk test (1965) using the distribution of W values (*P* < 0.1). Consequently, differences among treatments within a given species were analyzed by the Kruskal-Wallis *H* test, and followed, in the case of significant differences (*P* < 0.05), by multiple pairwise Mann-Whitney Ranked Sum tests (*U* tests; Sachs 1984). The correlation between foliar Fe and Mn concentrations (*n* = 22) was tested by nonlinear regression, and the correlations between foliar Fe concentration and chlorophyll concentration or *A* max (*n* = 6) by Spearman Rank correlation.

**Results**

**Toxicity symptoms and biomass production**

After 12 weeks of solution culture, all seedlings grown with high Mn²⁺ concentrations exhibited typical symptoms of Mn toxicity, i.e., small distinct dark leaf speckles and patchy leaf chlorosis, the symptoms being most severe at the highest Mn²⁺ concentration (1.2 mM Mn²⁺; MnR treatment), but were alleviated by an equimolar Mg²⁺ concentration (MnMg treatment; Table 2). No root damage was detected.
Length of the main shoot was unaffected by Mn²⁺ concentration, but in *Q. petraea*, the dry mass of the shoot axis (including twigs) was significantly reduced by the MnR treatment (Table 2). Total leaf mass (data not shown) as well as first-flush leaf mass did not differ among treatments. However, second-flush leaf mass was significantly reduced by MnR treatment in *Q. petraea*, but not in *Q. robur*. Dry masses of both coarse and fine roots were significantly decreased by the MnR treatment. The effect on coarse root mass was alleviated by an equimolar Mg²⁺ concentration in *Q. robur*, but not in *Q. petraea*. In the latter species, root length was also significantly decreased in the MnR treatment. In the MnR treatment, shoot axis, second-flush leaf and fine-root dry masses in *Q. petraea* were 31, 24 and 30% of control values compared with values of 62, 55 and 38% in *Q. robur*. Magnesium was less effective in alleviating reductions in dry mass and preventing necrosis in *Q. petraea* than in *Q. robur*.

**Microscopic analysis and element concentrations**

In cross sections of fine roots from MnR-treated seedlings, rhizodermal and cortical cells and some endodermal, central parenchyma and phloem cells exhibited dark inclusions (particularly apparent in *Q. robur*). No other symptoms of Mn toxicity in roots were observed. In MnR-treated seedlings of both species, much of the phloem of the petiole had collapsed (Figure 1). Transverse leaf sections of MnR-treated seedlings revealed dark inclusions, primarily in the palisade parenchyma cells, but also in upper epidermal and spongy mesophyll cells.

[Table 2](#) Symptoms of Mn toxicity (after 12 weeks) and growth parameters of seedlings of *Quercus petraea* and *Q. robur* in solution culture with different Mn²⁺ concentrations (for treatments, see Table 1). Growth variables (at the date of harvest): mean ± 1 SE; *n* = 10 (unless indicated otherwise). Different letters within a given species indicate significant differences among treatments (*P* < 0.05).

**Figure 1.** Cross sections of first-flush leaf petioles of *Quercus robur* seedlings grown for 22 weeks in nutrient solutions containing (a) adequate Mn²⁺ (0.0024 mM; control) or (b) high Mn²⁺ (1.2 mM; MnR). Sections were stained with gentian violet.
RESPONSES OF QUERCUS SPECIES TO EXCESS MANGANESE

(Figure 2a). In MnR-treated seedlings, transmission electron micrographs of the palisade parenchyma revealed electron-dense inclusions in the vacuoles (Figure 2c) that were absent from the palisade parenchyma of control plants (Figure 2b). The extent of these inclusions did not differ between species.

Manganese concentrations in the vacuoles (or cell lumina in the phloem) were much higher in MnR-treated seedlings than in controls (Figures 3a and 3b). In leaf cell walls of MnR-treated seedlings, Mn concentrations were significantly greater than in control plants, although they did not reach the concentrations observed in vacuoles, except in epidermal cell walls of Q. robur (Figures 3e and 3f). For seedlings in the MnR treatment, random measurements revealed that the upper and lower epidermis did not differ markedly in vacuolar or cell-wall Mn concentrations. In the vacuoles of the epidermis and the palisade parenchyma of Q. petraea, and in all four investigated tissue types of Q. robur, elevated Mn concentrations were accompanied by significantly elevated P concentrations (Figures 3c and 3d).

Foliar bulk Mn concentration increased with increasing Mn2+ concentration in the nutrient solution, reaching values of up to 39 mg gDM–1 (Figures 4a and 4b). In the MnA and MnR treatments, foliar Mg concentrations were significantly lower than in the control (Figures 4c and 4d). The MnR treatment induced Mg deficiency (as defined by van den Burg 1985) in second-flush leaves of both species, whereas the MnA and MnR

Figure 2. Cross sections of first-flush leaves of Quercus robur grown for 22 weeks in nutrient solutions. (a) Light-micrograph of a plant grown with high Mn2+ (1.2 mM; MnR) after staining with toluidine blue; transmission electron micrograph of palisade parenchyma cells of seedlings grown with (b) adequate Mn2+ (0.0024 mM; control) or with (c) high Mn2+ (1.2 mM; MnR).

Figure 3. Concentrations of (a, b) Mn and (c, d) P in the vacuoles or cell lumina, and of (e, f) Mn in cell walls of first-flush leaves of seedlings of Quercus petraea and Q. robur. Asterisks (*) indicate significant differences between the control (0.0024 mM Mn2+: filled bars) and MnR treatment (1.2 mM Mn2+: open bars) within a given tissue type (Epid., epidermis; Pal.p., palisade parenchyma; Sp.m., spongy mesophyll). Missing bars indicate values <1 mol m–3. The Mn concentrations of vacuoles and cell lumina (a, b) are scaled logarithmically. Values are means ±1 SE; n = 3.
treatments induced Mg$^{2+}$ deficiency in first-flush leaves of *Q. robur*. In both species and leaf cohorts, the addition of 1.2 mM Mg$^{2+}$ reversed the effect of the highest Mn$^{2+}$ concentration and restored leaf Mg concentration to the control value.

Foliar Fe concentrations were significantly reduced by all treatments with high Mn$^{2+}$ concentrations, with lowest foliar Fe concentration occurring in seedlings in the MnMg treatment (Figures 4e and 4f). In second-flush leaves, Fe concentrations were around or below the upper threshold that delimits the range of low Fe concentrations, i.e., the range in which trees react positively to Fe fertilization according to van den Burg (1985). Foliar Fe:Mn ratios ranged from 0.25 to 0.93 in control seedlings, whereas they were 0.03 or below in seedlings in the other treatments. In seedlings in the MnA, MnR and MnMg treatments, the Fe:Mn ratios were particularly low in second-flush leaves (0.01 or below). When all treatments were considered, there was a significant negative correlation between foliar Ca and K concentrations (Figure 5a).

High Mn$^{2+}$ concentrations significantly lowered foliar concentrations of Ca (except for first- and second-flush leaves of *Q. robur* in the MnA treatment) and K (except for second-flush leaves of *Q. petraea* in the MnR treatment and the second-flush leaves of *Q. robur*; Table 3). In *Q. robur*, Ca concentrations were below the normal range in second-flush leaves in the MnR treatment (the normal range is the range in which additional supply of the nutrient does not enhance growth; 5–11 mg Ca g$\text{DM}^{-1}$; van den Burg 1985), and K concentrations fell below normal (5–13 mg K g$\text{DM}^{-1}$; van den Burg 1985) in first-flush leaves in all high Mn$^{2+}$ treatments. There were no significant differences in foliar Ca and K concentrations between MnR and MnMg treatments.

**Gas exchange**

Eight weeks after the start of the experiment, $g_s$ was significantly reduced by high Mn$^{2+}$ concentrations (Figures 6a and 6b). In both leaf cohorts of *Q. robur*, the lowest $g_s$ values were found in the MnR treatment. In *Q. robur*, but not in *Q. petraea*, 1.2 mM Mg$^{2+}$ partially reversed the effect of the MnR treatment on $g_s$. A similar pattern was observed at 16 weeks, with two exceptions: (1) in *Q. petraea*, $g_s$ was lowest in the MnR treatment and did not differ from the control in the MnMg treatment; and (2) in *Q. robur*, $g_s$ was lowest in the MnMg treatment (Figures 6c and 6d).

At the end of the experiment, CO$_2$ assimilation parameters ($A_{\text{max}}, A_{\text{cap}}, V_{\text{cmax}}$ and WUE) were reduced in first-flush leaves by the high Mn$^{2+}$ treatments (MnR and MnMg; Figure 7). The differences were particularly large in *Q. petraea*; however, the limited number of measurements precluded statistical analy-
In both species, equimolar Mg$^{2+}$ did not alleviate the inhibitory effect of high Mn$^{2+}$ concentrations on CO$_2$ assimilation. There was a significant positive correlation between foliar Fe concentration and $A_{\text{max}}$ (Figure 5b).

In first-flush leaves of *Q. robur*, the MnR treatment significantly reduced the chlorophyll concentration (60 ± 14 versus 360 ± 60 mg m–2 in the control and 206 ± 43 mg m–2 in the MnMg treatment). In leaves of *Q. petraea*, MnR and MnMg treatments reduced chlorophyll concentrations (170 ± 50 and 179 ± 14 mg m–2 versus 379 ± 123 mg m–2 in the control), but

<table>
<thead>
<tr>
<th>Species</th>
<th>1st leaf flush Ca (mg g$_{\text{DM}}$–1)</th>
<th>2nd leaf flush Ca (mg g$_{\text{DM}}$–1)</th>
<th>1st leaf flush K (mg g$_{\text{DM}}$–1)</th>
<th>2nd leaf flush K (mg g$_{\text{DM}}$–1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Quercus petraea</em></td>
<td>17.2 ± 2.4 a</td>
<td>13.4 ± 1.5 a</td>
<td>10.6 ± 1.1 a</td>
<td>10.6 ± 1.0 a</td>
</tr>
<tr>
<td>Control</td>
<td>12.2 ± 0.9 b</td>
<td>8.4 ± 0.5 b</td>
<td>6.0 ± 0.7 b</td>
<td>6.5 ± 0.5 b</td>
</tr>
<tr>
<td>MnA</td>
<td>9.0 ± 0.9 c</td>
<td>5.2 ± 0.2 c</td>
<td>5.6 ± 0.5 b</td>
<td>10.8 ± 4.3 ab</td>
</tr>
<tr>
<td>MnMg</td>
<td>7.9 ± 1.2 c</td>
<td>7.0 ± 0.8 bc</td>
<td>5.1 ± 0.5 b</td>
<td>5.8 ± 0.6 b</td>
</tr>
<tr>
<td><em>Quercus robur</em></td>
<td>10.9 ± 1.2</td>
<td>11.4 ± 1.3 a</td>
<td>8.5 ± 0.4 a</td>
<td>8.2 ± 0.9</td>
</tr>
<tr>
<td>Control</td>
<td>9.2 ± 1.0</td>
<td>9.2 ± 0.9 a</td>
<td>4.4 ± 0.5 b</td>
<td>6.3 ± 1.1</td>
</tr>
<tr>
<td>MnA</td>
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<td>3.5 ± 0.5 b</td>
<td>3.5 ± 0.5 b</td>
<td>5.1 ± 1.3</td>
</tr>
<tr>
<td>MnMg</td>
<td>9.3 ± 0.9</td>
<td>5.2 ± 0.7 b</td>
<td>3.8 ± 0.5 b</td>
<td>5.1 ± 0.7</td>
</tr>
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</table>

In first-flush leaves of *Q. robur*, the MnR treatment significantly reduced the chlorophyll concentration (60 ± 14 versus 360 ± 60 mg m–2 in the control and 206 ± 43 mg m–2 in the MnMg treatment). In leaves of *Q. petraea*, MnR and MnMg treatments reduced chlorophyll concentrations (170 ± 50 and 179 ± 14 mg m–2 versus 379 ± 123 mg m–2 in the control), but
Figure 7. Photosynthetic parameters in seedlings of (a) *Quercus petraea* and (b) *Q. robur* grown in solution culture with different Mn2+ concentrations. Net photosynthesis (at light saturation and ambient CO2; *A*max), photosynthetic capacity (at saturating light and CO2; *A*up), maximum carboxylation rate (*V*max) and instantaneous water use efficiency (*A*max per unit transpiration; WUE) were measured in the first flush of control (0.0024 mM Mn2+; black bars), MnR (1.2 mM Mn2+; light-gray bars) and MnMg (1.2 mM + 1.2 mM Mg2+; dark-gray bars) treatments at the end of the experiment (means and ranges; *n* = 2).

the small number of replicates precluded statistical analysis.

Discussion

Toxicity symptoms and foliar bulk element concentrations

We observed typical Mn toxicity symptoms in leaves of plants grown in solution culture with high Mn2+ concentrations (0.24 or 1.2 mM; Horst 1988). Dark cellular inclusions are reported to contain oxidized insoluble manganese, the dark color deriving from oxidized polyphenols (Marschner 1995). The visible symptoms were less severe in MnA-treated than in MnR-treated seedlings. These weak to moderate toxicity symptoms, together with high foliar Mn concentrations (up to 20 mg gDM⁻¹), indicate that the investigated oak species were tolerant of high Mn2+ concentrations in the rooting medium. Plants sensitive to Mn2+ such as alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* (L.) Merr.) exhibit toxicity symptoms at foliar Mn concentrations of 0.2–0.4 mg gDM⁻¹, whereas Mn-tolerant plants such as *Vaccinium myrtillus* L. often display no visible symptoms of injury at foliar Mn concentrations of more than 1.5 mg gDM⁻¹ (Bergmann 1993).

The rate of Mn uptake versus solution concentration shows saturation kinetics (Rengel 2000). Foliar Mn concentrations in oaks subjected to the highest Mn2+ concentrations were high (up to 39 mg gDM⁻¹), but this is not unusual for plants growing in soil with high Mn concentrations. For example, in needles of 47-year-old Douglas-fir trees, Mn concentrations of up to 13 mg gDM⁻¹ have been found (Kaus and Wild 1998). Detached leaves of *Clusia multiflora* Kunth, an early successional species of tropical montane forests, accumulated up to 19 mg gDM⁻¹ Mn without symptoms of toxicity when kept in solutions containing 200 mM Mn (Chacon et al. 1998). Similarly, leaves of the Australian species *Austromyrtus bidwillii* (Benth.) Burrett (Myrtaceae), an Mn hyperaccumulator, contained up to 19.2 mg gDM⁻¹ Mn (Bidwell et al. 2002). Leaves of *Virotia neurophylla* (Guillaumin) Virot (Proteaceae) and *Gossia bidwillii* (Benth.) N. Snow & Guymer (Myrtaceae), grown in Oceanian tropical or subtropical rain forests, had Mn concentrations of 24.4 and 26.2 mg gDM⁻¹, respectively (Fernando et al. 2006a). In contrast, the maximum Mn concentration in leaves of 18 mature oak stands in northern Germany was 5.3 mg gDM⁻¹ (F.M. Thomas, data not shown), indicating that the Mn concentrations in the soils of these stands do not give rise to foliar concentrations as high as those found in our study.

Decreased foliar concentrations of Fe and Mg in response to high Mn2+ concentrations in the nutrient solution, were also observed in *Acer saccharum* seedlings (McQuattie and Schier 2000). The reduction in foliar Mg and Fe concentrations is caused by inhibition of Mg and Fe uptake through roots by excess Mn2+ (Mukhopadhyay and Sharma 1991, Bundt et al. 1997, Lidon 2002), which can result in Fe and Mg deficiency. In our study, Fe deficiency in leaves of plants grown with excess Mn2+ supply was indicated by Fe:Mn ratios of less than 0.03. The Fe:Mn ratios of leaves of deciduous woody species that exhibit no indication of Fe:Mn imbalance are distinctly higher than 0.1 (1.4–2.0 in *Populus* spp. and *Salix monticola* Bebb; Barrick and Noble 1993).

Excess Mn2+ in the rhizosphere inhibits the uptake not only of Fe and Mg, but also of Ca and K (e.g., Alam et al. 2003). In addition, excess Mn in plant tissues affects the translocation of Ca from roots to shoot by impairing basipetal transport of indole acetic acid, which is coupled with acropetal countertransport of Ca (Marschner 1995). These effects of excess Mn2+ were reflected in foliar Ca and K concentrations, particularly in *Q. petraea*. The lack of significant differences among treatments in Ca concentrations of first-flush leaves, and in K concentrations of second-flush leaves of *Q. robur* indicates that uptake and translocation of Ca and K were less affected in this species than in *Q. petraea*. The similarities in foliar Ca and K concentrations between MnR and MnMg treatments demonstrate that the Mg2+ supply in the MnMg treatment did not result in a reduced uptake of Ca or K via ion competition at the absorption sites of roots.

Manganese distribution among tissues and cell compartments

Energy dispersive X-ray analyses revealed that the epidermis (vacuoles and, in *Q. robur*, cell walls) of plants exposed to the
highest Mn²⁺ concentration had higher Mn concentrations than the palisade parenchyma and the spongy mesophyll. However, the number of replicates was low and the differences among tissue types were not significant. High epidermal Mn concentrations were also found in needles of Douglas-fir seedlings (Dućić et al. 2006). Our results contrast with those of Fernando et al. (2006a), who showed that, in leaf tissues of tropical and subtropical plants with high foliar Mn concentrations, excess Mn is primarily stored in photosynthetically active palisade parenchyma. In the epidermis, palisade parenchyma and spongy mesophyll of oaks supplied with high Mn²⁺, vacuolar Mn concentrations were higher than in cell walls by about one order of magnitude (except in Q. robur in which epidermal cell walls had high Mn concentrations). The vacuolar Mn concentrations were even higher in our study than in leaves of field-grown Gossia bidwillii, an Mn hyperaccumulator (up to about 600 mM; Fernando et al. 2006b). We found that the vacuoles of the epidermis and mesophyll tissue were the largest depositories of Mn. The elevated P concentrations in plants supplied with high Mn, except for the spongy mesophyll of Q. petraea, could be due to polyphosphate associated with Mn. Immobilization of Mn as Mn-polyphosphate granules is a mechanism of Mn tolerance in the vegetative parts of the lichen Lecanora conizaeoides Nyl. ex Cromb. (Paul et al. 2003). In the investigated oaks, however, only a minor fraction of Mn could be associated with polyphosphates, because vacuolar Mn concentrations were much higher than those of P. Our finding that Mn accumulated mainly in the vacuoles contrasts with results obtained with Douglas-fir, in which cell walls were the preferential site of Mn accumulation (Dućić et al. 2006). The accumulation of Mn in the vacuole may be related to high Mn tolerance in expanding tissues (Horst 1988) and to the low susceptibility of the investigated oak species to high Mn. An additional means of increasing Mn tolerance could be dilution of foliar Mn by translocation via the phloem and possibly its exudation or leaching from roots (cf. Bergmann 1993). Significantly increased Mn concentrations in phloem lumina of oak seedlings grown with high Mn²⁺ could be indicative of increased Mn translocation. This mechanism was also suggested by a ⁵²Mn-labeling study in Douglas-fir seedlings as an explanation for the different susceptibilities of two Douglas-fir subspecies to increased Mn concentration in the rooting medium (Dućić et al. 2006). However, the relatively high Mn concentrations accumulated in the leaf tissue of oaks investigated in our study, suggest that translocation of Mn from leaves accounts for only a small portion of Mn uptake, and thus, would not be a key tolerance mechanism.

Physiological responses

To be deposited in the vacuole, Mn must pass through the cytoplasm, where it can interfere with physiological processes. In our study, such interference was indicated by decreases in Amax and gₛ. Photosynthetic capacity shows a similar decrease in response to excess Mn (Lidon et al. 2004). However, the concomitant reductions in Vₘₐₓ and instantaneous WUE suggest that impairment of net photosynthesis and photosynthetic capacity was not primarily caused by a reduction in gₛ, but by an impairment of the photosynthetic apparatus (if gₛ alone had been reduced, an increase in instantaneous WUE would have been observed because stomatal closure confines the diffusion of H₂O to a relatively greater extent than that of CO₂; cf. Lamberts et al. 2006). Besides impairing chlorophyll synthesis (Marschner 1995), excess Mn inhibited the Fe uptake needed for synthesis of chlorophyll precursors. Consequently, chlorophyll concentrations, and photosynthetic activity of oak seedlings subjected to high Mn concentrations, were reduced, and Aₘₐₓ declined with decreasing foliar Fe concentrations (chlorophyll concentrations and photosynthetic parameters were measured in only a small number of plants at the end of the experiment). Decreased chlorophyll concentrations and photosynthetic CO₂ exchange rates were measured in leaves of Acer saccharum that exhibited increased Mn concentrations and lowered Mg concentrations due to edaphic conditions and were related to decline symptoms (St. Clair et al. 2005). Corresponding with our results, in nine evergreen and deciduous temperate tree species exposed to excess Mn²⁺, the relative change in gₛ, photosynthetic CO₂ exchange and electron transport rate (control versus treatment with high Mn²⁺ supply) was negatively correlated with foliar Mn concentration (St. Clair and Lynch 2005). Although the supply of additional Mg²⁺ raised the foliar Mg concentrations to control values, photosynthesis, and usually gₛ, remained below control values. This finding indicates that photosynthesis might have been impaired more severely by low foliar concentrations of Fe than by decreased concentrations of Mg.

Biomass partitioning and field performance

Damage to phloem as seen in oak seedlings exposed to excess Mn²⁺ has also been observed in seedlings of Picea abies (Keil et al. 1986) and Acer saccharum (McQuattie and Schier 2000). Collapsed phloem in the petioles may be associated with Mg deficiency induced by Mn, as supported by phloem collapse in needles of Mg-deficient P. abies (Fink 1991, Puech and Mehne-Jakobs 1997). We hypothesize that this damage— together with the reduction in CO₂ assimilation by high Mn and Mn-induced Fe deficiency—resulted in a lowered export of photosynthesize from first-flush leaves to actively growing plant organs. This explains the observed decrease in coarse and fine root biomass in oak seedlings subjected to 1.2 mM Mn²⁺. Circumstantial evidence supporting this hypothesis includes: (1) the correlation between foliar Mn and Fe concentrations; (2) the correlation between foliar Fe concentration and Aₘₐₓ; (3) the observed phloem damage; and (4) the reduction in root biomass in plants grown with excess Mn. Thus, the decrease in biomass production and length increment of roots results from a reduced allocation of photosynthesize, rather than from direct damage by excess Mn. Biomass production and length increment were more severely affected by Mn in roots than in shoots, although shoot gas exchange was affected by Mn. On the basis of light-microscopy, we could not determine whether the extent of damage to phloem function differed between oak species. If the phloem function of Q. petraea was more severely affected, it would explain the significant reduction in
second-flush leaf mass and the significantly reduced root length, which was not found in \textit{Q. robur}. Less impairment of phloem function in \textit{Q. robur} could be due to the greater effectiveness of Mg\textsuperscript{2+} in maintaining phloem stability. This would also explain why Mg\textsuperscript{2+} alleviated Mn toxicity symptoms and, in part, growth reductions in \textit{Q. robur} only.

In conclusion, high Mn\textsuperscript{2+} impaired growth more severely in \textit{Q. petraea} than in \textit{Q. robur}. However, differences between species became apparent only at the highest external Mn\textsuperscript{2+} concentration. At a lower Mn\textsuperscript{2+} concentration, representing the upper range of solutions from acidic forest soils (0.24 mM), growth of both oak species was unaffected. Under these conditions, foliar Mn concentrations exceeded the highest Mn concentration measured in leaves from mature oaks growing in near-natural stands. Therefore, Mn\textsuperscript{2+} concentrations in the field, under conditions other than strongly reducing ones, are unlikely to affect the performance or distribution of these species. Further supporting arguments are the duration of stress (at some sites, high Mn\textsuperscript{2+} concentrations in the soil solution occur only after acidification pulses or temporary waterlogging) and the ability of silicon to substantially increase the Mn tolerance of plant tissue (Horst 1988)—silicon was not supplied with the nutrient solutions in this study, but might be taken up in significant amounts from the soil in the field.

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