Ecotypic differentiation of mid-Atlantic *Quercus* species in response to ultramafic soils

Jerry Burgess^{A,E}, Katalin Szlavecz^A, Nishanta Rajakaruna^{B,C} and Christopher Swan^D

^ADepartment of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, MD 21218, USA.

^BCollege of the Atlantic, 105 Eden Street, Bar Harbor, ME 04609, USA.

^CUnit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa.

^DDepartment of Geography & Environmental Systems, University of Maryland, Baltimore County, Baltimore, MD 21250, USA.

^ECorresponding author. Email: jerry.burgess@jhu.edu

Abstract. Spatial heterogeneity of soil conditions combined with intraspecific variation confer site-specific edaphic tolerance, resulting in local adaptation and speciation. To understand the geoecological processes controlling community assembly of woodland tree species on serpentine and mafic soils, we investigated resource gradients and provenance (geographic area of propagule collection) as variables affecting typical representative upland oak (*Quercus*) species distribution. Accordingly, we conducted a year-long reciprocal transplant experiment in the greenhouse with serpentine and mafic soils, using seedlings of five oak species (*Quercus marilandica*, *Q. stellata*, *Q. montana*, *Q. michauxii* and *Q. alba*). All seedlings, regardless of provenance or soil depth, displayed more robust growth in the mafic soils. Soil depth was an important determinant, with all species exhibiting increased growth in the deeper-soil treatments. Fitness surrogates such as stem height, relative growth rate, and leaves per plant were greater when seedlings were grown in their home soil than when they were grown in the non-resident soil, suggesting an ecotypic effect. Mean stomatal conductance and stem growth were positively correlated with soil depth in all treatments. Taken together, the study showed provenance-specific growth responses of oak seedlings to soil type and depth, providing a better understanding of the mechanisms controlling species assembly in woodland communities.

Additional keywords: edaphic, intra-specific variation, local adaptation, reciprocal transplant, seedling growth, serpentine.

Introduction

Habitat specialisation can have significant evolutionary consequences, often contributing to local adaptation (Leimu and Fischer 2008; Yost *et al.* 2012; Bieger *et al.* 2014) and speciation (Rajakaruna 2004; Kay *et al.* 2011). Adaptive differentiation in response to distinct ecological pressures has long been recognised as a driver of species diversification (Darwin 1859; Clausen *et al.* 1940; Ågren and Schemske 2012). Although Darwin clearly implicated the contribution of adaptation to the process of speciation, recent studies have demonstrated how both divergent natural selection and ecological speciation along environmental gradients can affect phenotypic change and drive the evolution of new species (Linhart and Grant 1996; Geber and Griffen 2003; Kawecki and Ebert 2004; Schluter 2009; Yost *et al.* 2012).

Within the same climatic region, soils are perhaps the most important and least understood among the many factors influencing the ecology and evolution of plant species (Kruckeberg 1986; Rajakaruna 2004). Edaphic stresses such as salt or heavy metal toxicity, nutrient deficiency, or poor physical properties challenge

plant growth and development (O'Dell and Rajakaruna 2011). Edaphic environments are highly heterogeneous and vary over many spatial scales affecting the species' ecology and fitness (Lechowicz and Bell 1991; Wardle *et al.* 2004; van der Putten *et al.* 2004; Yost *et al.* 2012). A strong soil geochemical gradient, derived from geologic juxtaposition of edaphic extremes, can act as an agent of divergent selection resulting in ecotypic differentiation (Ettema and Wardle 2002; Rajakaruna *et al.* 2003; Baythavong and Stanton 2010). Such edaphic extremes occur in woodlands and forests of the mid-Atlantic piedmont because of geologic and pedologic discontinuities, resulting in nutrient-poor or chemically imbalanced, shallow ultramafic soils adjacent to well developed (deeper) and nutrient-rich mafic soils. The two soil types have marked differences in calcium (Ca)+magnesium (Mg):Mg ratios, which are diagnostic differentiators of mafic and ultramafic soils in the Piedmont (Shaw *et al.* 2001; Burgess 2013). Plant species on these resource bases are likely to employ different mechanisms to cope with the dry, nutrient imbalanced ultramafic soils compared with the more mesic and fertile mafic soils.

Extreme habitats resulting from edaphic conditions are areas of intense environmental filtering, often leading to a unique set of adaptations suited to the local conditions (Harrison *et al.* 2006; Cornwell and Ackerly 2009; Siegwart Collier and Mallik 2010; Yost *et al.* 2012). Often listed as a model system to study evolution and ecotypic adaptation (Harrison and Rajakaruna 2011), ultramafic environments have been the focus of numerous studies on adaptive evolution (Kay *et al.* 2011; Anacker 2014, and references therein). Environmental filtering is extensive on ultramafic soils because they frequently have low water-holding capacity, contain elevated concentrations of heavy metals such nickel (Ni), chromium (Cr) and cobalt (Co), and are often deficient in essential macronutrients (O'Dell and Rajakaruna 2011). Soil depth and correlated factors such as water-holding capacity have also been suggested as a driver of community assembly in some serpentine areas of the mid-Atlantic piedmont (Barton and Wallenstein 1997; Burgess 2013). Soil depth and moisture also correlate with productivity and resource availability in serpentine habitats (Whittaker 1954; Davies *et al.* 2007; Moore and Elmendorf 2011). The importance of these two factors have also been demonstrated in invasive species success in some grasslands (Selmants *et al.* 2012) and questioned as a driver of invasion success in others (Schedlbauer and Pistoia 2013).

In the eastern North American hardwood forests, the largest tree genus, *Quercus* (oak), is abundant and also known as a foundation species (Fralish 2004; Ellison *et al.* 2005; McShea *et al.* 2007). However, the future of oak forests is uncertain because forests are affected by events such as insect herbivory and pathogen introductions (Grünwald *et al.* 2012; Bereczki *et al.* 2014). Other impacts include stressors such as development and extensive logging (Nowacki and Abrams 1992; Lorimer 1993; Abrams 2003; Johnson *et al.* 2009) and species invasions (Tyndall 1992; Burgess *et al.* 2015). To address oak performance in serpentine environments, we explored resource gradients and provenance as variables affecting typical representative upland oak species assembly by examining their carbon-allocation strategies and stomatal conductance. We conducted a year-long reciprocal transplant experiment on ultramafic and mafic soils, using seedlings of five oak species (*Quercus marilandica* Muenchh, *Q. stellata* Wangenh, *Q. montana* Willd, *Q. michauxii* Nutt and *Q. alba* L.) found in the mid-Atlantic piedmont. Previous studies of populations of forest trees have shown that populations can survive outside the home site and even thrive in situations void of competition or resource limitations (Heikinheimo 1949; Beuker 1994; Rehfeldt *et al.* 1999, 2002; Shutyaev and Giertych 2000; Reich *et al.* 2003). However, intra- and interspecific competition limit the distribution and reproductive success of individual genotypes, with relative fitness often being higher at the local or resident site (Clausen *et al.* 1940; Kawecki and Ebert 2004; Savolainen *et al.* 2007). In a meta-analysis of plant populations, Leimu and Fischer (2008) found that local plants outperformed non-resident plants at their site of origin 71% of the time. Contradictory data from Carter (1996) showed that 8 of 10 studies of tree species in the eastern USA have shown growth optima in areas north of their current range and would have higher fitness if grown in a cooler climate. Indeed, the literature contains examples of populations that have a lower relative fitness than do

non-resident transplants (Rice and Mack 1991; Hereford and Winn 2008; Bieger *et al.* 2014) and, in the work of Leimu and Fischer (2008), it was shown that in pair-wise comparisons of local adaptation, local plants outperformed non-resident plants only 45.3% of the time. Most local-adaptation studies focus on short-lived herbaceous species; thus, work on long-lived species such as trees is important to evaluate whether similar processes are operating. Wright (2007) investigated serpentine adaptation in a 36-year experiment using *Pinus ponderosa* and concluded that adaptation was evident during longer time spans (>2 years). Branco (2009) documented significant physiological differences between *Quercus ilex* seedlings collected from serpentine and non-serpentine site, but documented no home-site advantage suggestive of local adaptation.

In the present study, we examined the effect of soil type derived from serpentinite, (ultramafic) and gabbro (mafic) parent material and soil depth on growth of resident (native or local) and non-resident oak seedlings. We hypothesised that owing to their unique adaptations, these oak seedlings will exhibit increased growth rates in mafic and home-site soils. Implicit in this hypothesis is that native serpentine species are more tolerant of shallow, water-stressed and nutrient-deficient and imbalanced conditions than are adjacent non-serpentine species. Species commonly found on harsh substrates will show adaptations to xeric conditions such as strong stomatal control able to maintain gas exchange during stressful conditions and increased belowground biomass allocation (Cornic and Massacci 1996; Hermans *et al.* 2006). We utilised a reciprocal transplant (introducing organisms from each of two or more environments into the others) experiment to examine whether the resident genotypes exhibit higher relative fitness than do non-resident genotypes. Local adaptation was evaluated using morphological and physiological traits (growth rate, biomass production/allocation) as imperfect fitness surrogates. Elemental concentrations in the foliar tissue were used to evaluate possible coping strategies for seedlings grown in high metal-content serpentine soils.

Materials and methods

We employed a factorial design using five tree species, with the main factors being soil type nested within varying soil depths. Seeds from five species of oak (*Q. alba*, white oak; *Q. marilandica*, blackjack oak; *Q. michauxii*, basket oak; *Q. montana*, chestnut oak; and *Q. stellata*, post oak) were used because of their prevalence on serpentine and (or) mafic soils. Throughout the mid-Atlantic, *Quercus* species can be found on a variety of soil types; typically, *Q. marilandica* and *Q. stellata* predominate on xeric sites or soils derived from serpentinite rock, whereas the widely distributed *Q. alba*, *Q. montana* and *Q. michauxii*, among others, will occur on both soil types, although at a considerably lower basal area and density on ultramafic soil. Seeds (resident and non-resident) were planted within six treatments that included three levels of soil depth (shallow, 15 cm; medium, 30 cm; and deep, 60 cm) and two soil treatments: serpentine (ultramafic) and gabbro (mafic). These depths were chosen as representative of soil depths in serpentine savannas, with the deeper treatment more typical of mature woodlands/forests developed on these soils (Burgess *et al.* 2015).

Seed collection and preparation

Approximately 1500 acorns were hand-collected in 2008 from three areas in the Piedmont of Maryland (39.387055 N, 76.653156 W; 39.641058 N, 76.363564 W; and 39.704034 N, 76.190658 W). Not all oak species were present at each collection site and the number of maternal trees varied per site; thus, the effect of maternal tree on seedling response was not studied. All acorns from each population (species and provenance) were pooled together. Cups (woody involucre) of the acorns were removed, and undamaged acorns (e.g. those without insect damage) were placed in plastic bags containing peat moss. Acorns were cold-stratified at 4 °C for 90 days and then immersed in water for 24 h. Acorns that floated were discarded; remaining acorns were placed in flats of moist vermiculite-based potting soil for germination on 1 January 2009. After separation from the pericarp, single acorns ($n = 414$) were weighed individually before sowing. After germination (emergence and development of the cotyledons), randomly chosen acorns ($n = 280$) were weighed and transplanted at 3-cm depth within a growth tube.

Soils and growth tubes

Approximately 600 L of ultramafic and mafic soils were collected in the Maryland piedmont from the upper 15 cm of the soil profile (grasslands for ultramafic soils and beneath a closed canopy for mafic soils). Mafic soils are of the Neshaminy series, forming very deep (>80 in) fertile farmland. Ultramafic soils are of the Chrome series, which are well drained, shallow (<30 in) and relatively infertile for crops. Soils were extensively homogenised, air-dried and sieved through a 2.0-mm screen to remove large debris. A subset of the homogenised soils was collected for analysis. Soil pH was measured with a glass electrode in a soil–water slurry of 20 g soil in 20 mL of water. Samples were also evaluated for moisture retention (5-point scale), particle-size distribution, total acid-extractable metal analysis by microwave assisted HNO_3 acid digestion using inductively coupled plasma–atomic emission spectroscopy (ICP–AES), combustion analysis of total carbon (C) and nitrogen (N), NH_4Cl -extractable bases aluminium (Al), Ca, Mg, potassium (K), sodium (Na) analysis by ICP–AES and cation exchange capacity (CEC). Analyses were performed at the Cornell Nutrient Analysis Laboratory Ithaca, New York.

Experiments were conducted for one growth season with seedlings in cylindrical growth chambers (5-cm-diameter, Blue-X[®], Enterprises, Inc., Sacramento, CA, USA). The respective soils were placed into the grow tubes utilising the following three experimental soil depths: 15 cm, 30 cm and 60 cm, with four replicates for each of the seed types amounting to 280 growth tubes. Forty of these replicates were taken for early harvest. Additional replicates were limited by seed supply and available space. The bottom of each tube contained respective parent regolith down to 3-cm depth, so as to facilitate drainage, and a pot supported each tube. Grow tubes were watered to saturation just before seedling placement.

Greenhouse conditions and measurements

A minimum 12-h photoperiod was maintained with a combination of indoor and natural lighting. Natural lighting was supplemented with cool-beam, full-spectrum lamps to increase the photoperiod

during the early growing season and to offset shading owing to greenhouse restrictions. Light conditions for the growing area were characterised by measuring photosynthetic photon flux rates over the 400–700-nm wavebands. Light conditions were uniform across the experimental area, with only minimal light obstruction from greenhouse support structures. Environmental conditions in the greenhouse varied according to daily weather, ranging as follows: photon flux at maximum chamber height of 60 cm, 230–1700 $\mu\text{mol m}^{-2} \text{s}^{-1}$, day temperature 18–40 °C; and relative humidity 40–70% day and night. Grow tubes were watered at 200 mL week⁻¹. Soil moisture was monitored using a ThetaProbe[®] (Dynamax, Inc., Houston, TX, USA) soil moisture sensor. At harvest, the oven dry mass (60 °C for at least 48 h) of roots, stems and leaves was determined, as well as the maximum rooting depth in the deeper tubes. The harvest data from March (40 samples after 21 days) and November 2009 (231 surviving seedlings) were used to calculate relative growth rate (RGR) and biomass allocation (root : shoot) values. RGR was calculated using the classical approach of Hunt (1990) where RGR is calculated from samples of individuals from the same cohort at two points in time (Evans 1972). A RGR was calculated for each seedling, using the following equation from Hunt (1990):

$$r = \frac{\ln(\bar{w}_2) - \ln(\bar{w}_1)}{t_2 - t_1},$$

where w_1 and w_2 are the plant dry mass across the harvest interval ($t_2 - t_1$).

Monthly parameters included volumetric soil moisture, germination, survival, growth (height, number of leaves, leaf length and width) humidity, photon flux, temperature and stomatal conductance. Stomatal conductance (in $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) was measured on four occasions (30 May 2009, 20 June 2009, 20 July 2009 and 19 August 2009) with a steady-state diffusion leaf porometer (SC-1 Leaf porometer, Decagon Devices, Pullman, WA, USA). Stomatal conductance (g_s) measurements were taken from the abaxial surfaces of fully expanded upper leaves.

Growth rates and plant tissue elemental analysis

For elemental analysis, leaves from the uppermost recently matured leaves were harvested during the growing season (23 July, 1 per seedling) and at the conclusion of the experiment (7–9 November). *Quercus marilandica* and *Q. stellata* leaf tissue masses were sufficient for seed-provenance and soil-type treatments to be analysed separately. *Quercus montana*, *Q. alba* and *Q. michauxii* had insufficient leaf sample mass for elemental analysis from some treatments and, thus, leaves of these species were grouped (mixed) by soil type. Leaf tissue was homogenised in an agate ball mill and then analysed via combustion for total C and N and by ICP for major elements as well as arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), Co, Cr, lithium (Li), Ni, lead (Pb), antimony (Sb), selenium (Se), silicon (Si), strontium (Sr), thallium (Tl), vanadium (V). Analysis was performed by Ohio State University STAR Laboratory, Wooster, OH, USA, using nitric acid microwave digestion.

Treatments were maintained through November 2009. On harvest, first, plant heights were measured, then the entire shoot

was cut off at soil level and separated into stems and leaves and roots. To extract roots from tubes, tubes were turned on their sides inside of a soil-washing container with 1-cm screen, the outermost liner was carefully removed and then the inner liner was peeled away from the soil and the depth of the root system (maximal rooting depth) was determined. This was measured only in the 60-cm-depth tubes as roots in the other treatments all extended to the maximum depth. All tissues and roots were dried separately at 60 °C for 7 days and massed. Specific root lengths (SRL) were calculated by dividing total root length (m) by root biomass (g) and root : shoot ratios were simply the dry weight for roots divided by dry weight of the aboveground part.

Because mycorrhizal root associations as well as other microbes are often important symbionts for nutrient uptake and plant performance, a bioassay was conducted at the conclusion of the experiment. A subset of soil samples was composited by soil type and assayed for active and total fungal and bacterial biomass, protozoa or nematode biomass and mycorrhizal colonisation. Sample combinations were randomised and watering was controlled to avoid mixing of microbial communities in the greenhouse. Analyses were performed by Soilfood Web Laboratory Corvallis, OR, USA. Active bacteria and fungi were counted by the fluorescein diacetate staining (FDA) method (Ingham and Klein 1982). Total bacterial numbers and the length of fungal hyphae were determined by phase-contrast microscopy and converted to micrograms of biomass per gram of dry soil.

Statistical analysis

The experiment was implemented and analysed as a completely randomised factorial design, using five species with two soil types, two provenances and three soil depths. For growth analyses, only seedlings surviving for the entire experimental period were included. Relationships between seed mass and growth parameters were analysed by regression analysis, and the curve equation and the value of R^2 were used to determine the correlation strength. Principal component analysis (PCA) on transformed data was used to evaluate seedling leaf elemental associations with the environmental factors (harvest date, species, provenance and soil type). Differences between soil types and foliar metal content were evaluated using Student's *t*-tests. Foliar elemental transfer efficiencies were evaluated with separate ANOVAs for each element, using harvest date, provenance and soil type as factors. Factorial ANOVAs were performed to test for differential responses and interactive effects between the response variables tissue nutrient/elemental concentrations, carbon allocation responses (stem height, biomass, RGR and leaf number) and the experimental factors soil type, provenance, species and soil depth. Repeated-measures ANOVA was used for stomatal-conductance responses, and using the corresponding moisture data for four census periods as a covariate. ANOVAs were followed by Tukey's (chosen because of an uneven number of replicates) multiple pairwise comparison to determine which species treatments had different biomass or growth-rate responses. To meet the assumptions of PCA or ANOVA, response-variable data were log-transformed as follows: natural log for foliar chemistry; $\log_{10}$ for stem height, biomass, root : shoot; and $\log_{10} + 1$ for RGR. To analyse rooting-depth

data, only the deep treatment was used, because plant roots typically grew to the full length in the more shallow treatments. Correlations were evaluated with the non-parametric Spearman rank correlation analysis because of deviation from normality and lack of any linear relationship of the leaf elemental data to other parameters. A significance level of $\alpha=0.05$ was used for the statistical tests, and a *P*-value of <0.05 indicated a significant difference. All errors are reported as standard error. All analyses were performed in R v.3.0.1 R (R Core Team 2013).

Results

Soils from the two provenances were loams (ultramafic), with 36–38% sand, and silty-loams (mafic), with 18–20% sand. Soil–water retention (0.10, 0.33, 1.00, 3.00 and 15.00 bars) was similar, except the mafic material had a higher matric potential (22.38% versus 20.03%) at 3 bars.

Soil pH values were effectively biologically neutral, with 6.40 $\pm$ 0.02 ($n=10$) and 6.85 $\pm$ 0.04 ($n=10$) for mafic and serpentine soils, respectively. Soil organic C, total N and CEC were similar (Table 1). Notable differences either in the extractable bases or the total metal concentrations included a nearly a four-fold difference in Mg (elevated in ultramafic soils), a four-fold difference in Ca (elevated in mafic soils), a 8–10-fold difference in Cr, Co and Ni (elevated in ultramafic soils), a two-fold difference in Al (elevated in mafic soils) and a two-fold difference in iron (Fe) content (elevated in ultramafic soils). The parent material discrimination metric (Ca + Mg) : Mg espoused by Shaw *et al.* (2001) yielded marked differences of 0.23 for ultramafic soils and 5.63 for mafic soils, using exchangeable base data and a factor of 10 difference using total metals (0.07 for ultramafic and 0.61 for mafic soils). Thus, these soils have similar nutritional content (C, N, Na, phosphorus (P), K) but with an imbalance in the Ca : Mg ratio and water-holding capacity.

The structure of the soil microbial community was similar between the two soils, with both being dominated by bacterial biomass and having a fungi : bacteria ratio less than unity (Table 2). ANOVA results suggested a significant ($P<0.05$) relationship between soil moisture content and stomatal conductance with soil depth during all sampling periods.

Differences in seed mass

Initial fresh seed mass significantly differed among the *Quercus* species (ANOVA $F_{9,749}=873.7$, $P<0.001$; Table 3). Only three species had acorns with a statistically significant difference in seed mass between soil types (*Q. marilandica*, *Q. montana* and *Q. alba*). *Quercus marilandica* and *Q. stellata* had the lowest masses of ~1.1–1.6 g, with no significant difference for the pair on the basis of provenance. *Quercus montana* and *Q. alba* had larger seeds (~4 g fresh mass), with both species showing significant differences in mass on the basis of provenance (smaller seeds on serpentine substrate). The largest seeds belonged to *Q. michauxii* at ~10 g, with larger seeds occurring in ultramafic soils. The relationship between seed mass and RGR ($r_s=0.223$, $P=0.007$) and stem height ($r_s=0.493$, $P=0.001$) for all data points was considered using Spearman's rank correlation, which suggested a weak positive relationship between initial acorn mass and ultimate seedling performance.

Foliar elemental content

Concentrations of extractable elements in leaf tissue varied considerably and significantly (ANOVA, $P < 0.05$) between the July and August harvest dates. Mean concentrations of heavy metals in leaf were significantly (one-way ANOVA,

Table 1. Total and extractable metal concentrations, pH and chemical soil characteristics for the two experimental soils

Parameter	Serpentine soils	Mafic soils
Soil characteristic		
N (%)	0.35	0.32
C (%)	3.91	3.62
pH	6.85	6.40
NO ₃ + NO ₂ (mg kg ⁻¹)	49.02	44.76
NH ₄ -N (mg kg ⁻¹)	6.58	10.87
Cation exchange capacity (cmol kg ⁻¹)	28.33	21.80
Concentration of an extractable base (mg kg ⁻¹)		
Sodium	46.47	13.15
Magnesium	1087.11	243.99
Aluminium	3.98	8.34
Calcium	241.08	1310.10
Manganese	3.43	15.08
Iron	7.39	1.74
Zinc	5.81	8.21
Total metal concentration (mg kg ⁻¹)		
Sodium	64.18	57.04
Magnesium	15 970.00	2619.00
Aluminium	7057.00	13 830.00
Phosphorus	614.10	584.50
Potassium	480.90	281.90
Calcium	561.20	1314.00
Chromium	369.80	31.48
Manganese	1332.00	942.70
Iron	40 720.00	18 400.00
Cobalt	104.90	13.36
Nickel	1155.00	18.04

$P = 0.05$) different for all but Cr and Fe on the basis of soil treatments (Table 4). The variation in foliar content was similar for C (44.3–49.1%) across sampling dates, whereas for N (0.6–2.6%), there was a significant ($P = 0.01$) difference, with a contraction in values during the November sampling. ANOVA using soil type as a factor and leaf trace-element concentration as the response variables showed significant ($P < 0.05$) differences for N, sulfur (S), P, Ca, Mg, manganese (Mn), Ni and zinc (Zn), with K falling just outside the threshold criteria ($P = 0.06$). These data were analysed using a principal-component analysis to reduce dimensionality and increase overall pattern recognition (Fig. 1). To identify the effect from the experimental factors on the extractable leaf elements, the correlations of the extracted vectors of each PCA axis with harvest date, soil type, provenance and species were assessed by regression analysis (Table 5). The first PCA axis explained 39% of the total variance and was significantly correlated with soil type and harvest date. The second axis was correlated with harvest date, whereas provenance and species were significantly correlated with the third principal axis. Leaf P, N, K and S loaded mainly on this first axis, leaf Ca and Mn mainly loaded on the second axis, which explained 27% of the data, and leaf Mg and Ni loaded on the third axis, explaining 16% of the data. The data are consistent with basic nutrients being sensitive to leaf harvest date (growth and abscission), with Mg content aligning with particular species or provenance.

To test for the strength of association of leaf element concentration between the two soil treatments, Spearman's rank correlation was conducted, showing that foliar concentration was strongly correlated between the two soil treatments ($r_s = 0.97$, $P = 0.001$, $n = 29$). Notable differences included higher leaf Ca in seedlings grown in mafic soils and higher Mg and Ni in typical serpentine soils, whereas P, K and Fe were more elevated in the ultramafic soils, perhaps owing to allochthonous input.

As is typical of ultramafic soils, leaf concentration was diagnostic of ultramafic soils for several elements (Ca, Mg, Ni and Cr). These elements are displayed in Fig. 2, which highlights

Table 2. Ratios and estimated biomass of total populations of different functional groups from composite soil samples

Units are $\mu\text{g g}^{-1}$ biomass from actual count data

Soil parent	Active bacteria ($\mu\text{g g}^{-1}$)	Total bacteria ($\mu\text{g g}^{-1}$)	Active fungi ($\mu\text{g g}^{-1}$)	Total fungi ($\mu\text{g g}^{-1}$)	Mycorrhizal colonisation ($\mu\text{g g}^{-1}$)	Active fungi to active bacteria ratio
Ultramafic serpentine	59.70	1179.00	30.70	659	Not detected	0.51
Mafic gabbro	48.80	1347.00	16.90	495	Not detected	0.35

Table 3. Statistical parameters for acorn ($n = 759$) mass of *Quercus* species

P -values (from ANOVA) represent comparisons of seed mass by species across their resident soil types. The multiple comparisons of means followed the Tukey's method. IQR, interquartile range

Seed species	n	Seed fresh smass (serpentine provenance)			n	Seed fresh mass (mafic provenance)			P -value
		Mean $\pm$ s.e.	IQR	Germination (%)		Mean $\pm$ s.e.	IQR	Germination (%)	
<i>Q. marilandica</i>	123	1.18 $\pm$ 0.19	0.27	94.3	64	1.22 $\pm$ 0.05	0.59	95.3	1
<i>Q. stellata</i>	70	1.14 $\pm$ 0.03	0.36	91.4	68	1.64 $\pm$ 0.03	0.34	94.1	0.07
<i>Q. michauxii</i>	58	10.39 $\pm$ 0.20	1.61	98.2	65	9.77 $\pm$ 0.22	2.32	93.8	0.01
<i>Q. montana</i>	63	4.21 $\pm$ 0.16	1.68	89.9	69	5.87 $\pm$ 0.11	1.25	85.7	<0.01
<i>Q. alba</i>	100	3.98 $\pm$ 0.08	0.89	65.6	79	4.64 $\pm$ 0.13	1.97	83	<0.01

Table 4. Foliar elemental concentration (in $\mu\text{g g}^{-1}$ for metals; in % for carbon (C) and nitrogen (N)) in oak leaves

Qs, *Q. stellata*; *Q. ma*, *Q. marilandica*; *Q. mo*, *Q. montana*; *Qa*, *Q. alba*; and *Qmi*, *Q. michauxii*; M, mafic soil chemistry; G, gabbro home site; U, ultramafic soil chemistry; S, serpentine home site; Mix, combined samples from both provenance locations; P, phosphorus; S, sulfur; K, potassium; Ca, calcium; Mg, magnesium; Al, aluminium; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc; Cr, chromium; Ni, nickel

Harvest date	Soil parent or provenance	Species	N	C	P	S	K	Ca	Mg	Al	Cu	Fe	Mn	Zn	Cr	Ni
July	M/G	<i>Qs</i>	1.9	47.2	1455.7	1081.7	11356.7	4075.8	2003.3	31.8	10.6	66.1	456.4	22.0	0.9	6.7
July	M/G	<i>Qma</i>	1.8	48.4	532.8	951.0	3906.5	3589.1	2256.9	33.6	4.3	67.6	89.0	14.4	0.9	8.0
July	M/S	<i>Qs</i>	2.0	47.2	1255.5	1200.6	8453.0	5134.2	2149.0	27.6	5.8	92.9	519.8	23.6	1.1	5.1
July	M/S	<i>Qma</i>	2.5	46.8	986.8	1682.6	6269.2	4272.8	2150.8	32.9	4.2	85.0	957.4	34.0	1.6	1.9
July	M/Mix	<i>Qmo</i>	2.4	45.1	961.5	1770.2	5075.1	16696.6	5142.7	27.5	5.1	98.0	1496.2	24.1	2.0	2.4
July	M/Mix	<i>Qa</i>	2.2	46.1	1111.8	1669.8	8775.1	10255.9	3568.0	11.1	4.9	58.2	1412.5	20.3	1.7	4.3
July	M/Mix	<i>Qmi</i>	2.6	46.0	712.7	1684.9	4588.7	13500.3	3293.7	37.1	5.8	102.7	1903.3	18.9	2.3	1.5
Nov.	M/G	<i>Qs</i>	0.8	46.7	387.4	866.4	1067.9	8210.5	2398.2	25.6	3.6	76.2	1037.7	18.5	1.4	11.2
Nov.	M/G	<i>Qma</i>	0.8	48.2	213.7	803.2	818.6	7724.6	5817.0	18.7	2.4	49.5	454.9	26.6	1.1	16.4
Nov.	M/S	<i>Qs</i>	0.9	46.5	356.6	939.2	2153.2	8312.4	2140.9	76.1	4.5	163.3	1858.9	30.6	3.2	7.5
Nov.	M/S	<i>Qma</i>	1.4	47.0	752.7	1284.4	4790.6	9362.2	1919.4	19.3	3.3	81.0	2271.2	49.5	2.3	1.5
Nov.	M/Mix	<i>Qmo</i>	1.6	44.3	569.9	1639.8	1563.9	21836.6	6357.4	93.6	4.0	203.3	3323.2	21.7	4.2	2.9
Nov.	M/Mix	<i>Qa</i>	0.9	46.5	562.7	1004.6	2498.9	10507.5	2127.7	42.9	3.2	65.0	2608.0	17.5	2.9	4.7
Nov.	M/Mix	<i>Qmi</i>	2.0	45.9	867.0	1624.1	2633.6	15711.6	3688.0	55.7	3.7	127.1	3853.4	27.3	3.9	2.1
July	U/G	<i>Qs</i>	2.1	48.1	656.5	1134.4	5100.0	9615.3	3448.2	18.8	5.3	56.0	326.9	14.0	0.7	15.4
July	U/G	<i>Qma</i>	1.8	48.4	584.0	1002.4	3889.6	8595.1	4622.1	30.6	4.5	70.5	137.4	16.0	0.8	8.9
July	U/S	<i>Qs</i>	2.0	47.9	550.6	1044.2	4049.5	4460.2	3999.2	24.6	3.7	63.7	110.6	13.3	0.7	7.8
July	U/S	<i>Qma</i>	1.9	47.9	550.5	1062.3	3303.9	3707.2	4843.9	21.8	3.1	145.1	142.4	13.7	0.9	11.1
July	U/Mix	<i>Qmo</i>	2.2	46.5	1013.0	1305.0	4501.3	6407.4	6460.3	45.1	3.8	118.0	379.2	14.7	1.4	13.1
July	U/Mix	<i>Qa</i>	2.0	46.9	987.3	1208.0	4570.6	5782.2	4195.8	21.4	4.8	95.7	719.7	13.3	1.4	18.6
July	U/Mix	<i>Qmi</i>	2.3	45.4	752.7	1363.1	3346.8	7701.5	11387.9	32.1	2.4	95.9	156.5	16.1	1.1	8.8
Nov.	U/G	<i>Qs</i>	0.7	46.7	263.9	795.7	1158.2	6990.0	4844.2	21.5	3.3	67.4	1044.4	17.6	1.4	12.0
Nov.	U/G	<i>Qma</i>	0.7	49.1	148.8	872.1	780.2	8178.4	5685.5	23.6	3.0	71.6	664.6	18.7	1.6	6.7
Nov.	U/S	<i>Qs</i>	1.0	47.3	193.4	870.5	1000.8	6176.5	3387.6	32.1	3.8	69.6	1055.2	17.0	2.3	38.9
Nov.	U/S	<i>Qma</i>	1.0	48.4	215.8	991.8	1203.2	6734.6	3801.1	30.4	3.7	85.6	2706.7	24.3	3.6	26.2
Nov.	U/Mix	<i>Qmo</i>	0.1	47.0	343.6	1013.2	1153.3	7701.2	5963.6	79.8	3.0	186.1	2040.1	12.1	3.5	26.4
Nov.	U/Mix	<i>Qa</i>	0.6	46.3	353.2	948.8	1880.5	7583.5	5820.1	24.7	2.9	58.9	828.4	12.4	1.7	17.9
Nov.	U/Mix	<i>Qmi</i>	0.7	46.3	575.7	1079.2	2106.0	6877.1	9499.3	52.0	1.9	121.0	138.8	13.4	1.4	8.9
	Mean	Ultramafic	1.5	47.3	513.5	1049.3	2717.4	6750.7	5568.5	32.7	3.5	93.2	746.5	15.5	1.6	15.8
	Mean	Mafic	1.3	46.6	766.2	1300.2	4567.9	9942.1	3215.2	38.1	4.7	95.4	1588.7	24.9	2.1	5.4

Fig. 1. Projection of the leaf elemental concentrations for all independent samples ($n=28$) on the two first axes of the principal component analysis (PCA).

the time factor or harvest date for *Q. stellata* and *Q. marilandica*. These two species are detailed here, because they are the primary serpentine savanna woody species and they have the most

complete foliar element dataset. Broadly speaking, across the entire growing season, the mean concentration of extractable Ca was lowest in *Q. marilandica* ($5896 \mu\text{g g}^{-1}$) and *Q. stellata* ($5622 \mu\text{g g}^{-1}$), whereas *Q. michauxii* ($10\,948 \mu\text{g g}^{-1}$) and *Q. montana* ($13\,160 \mu\text{g g}^{-1}$) exhibited a two-fold increase, with *Q. alba* ($8532 \mu\text{g g}^{-1}$) being intermediate, even though Mg concentrations were similar in the resident seedlings, decreasing slightly in the non-resident treatments (Table 4). Ni and Cr uptake was similar for the two *Quercus* species, whereas tissue concentrations of Cr were lower and those of Ni doubled for *Q. montana* and there was a five-fold increase in *Q. stellata* just before abscission fall harvest for the resident serpentine treatment. A two-way ANOVA was used to examine the effect of metal concentration on the basis of soil type and seed origin for the serpentine signature elements Ca, Mg, Cr, Ni as well as the Ca:Mg ratio. There was a significant interaction between the effects of seed provenance and soil type for Ca:Mg ($F(2,22)=3.426$, $P=0.049$), Ca ($F(2,22)=5.248$, $P=0.014$) and Ni ($F(2,22)=3.510$, $P=0.047$). Simple main effect analysis showed that Ca:Mg ($P=0.0001$), Ca ($P=0.0008$), Mg ($P=0.001$) and Ni ($P=0.0004$) were significantly related to soil type, whereas there were no differences in Cr concentration among the soil types ($P=0.161$), although

Table 5. The factor loadings of nine leaf elements on the first three principal component analysis (PCA) axes, and correlation coefficients between each PCA axis and each of three environmental factors

Asterisks mark the significant ($P < 0.05$) correlations between the PCA axis and the environmental factors ($n = 28$)

Parameter	Component 1	Component 2	Component 3
Factor			
Nitrogen	0.741	-0.489	0.147
Phosphorus	0.892	-0.241	0.078
Potassium	0.841	-0.416	-0.130
Sulfur	0.794	0.354	0.380
Calcium	0.215	0.869	0.340
Magnesium	-0.310	-0.032	0.865
Manganese	0.100	0.915	-0.145
Nickel	-0.712	-0.225	-0.082
Zinc	0.417	0.475	-0.528
Total variation explained (%)	39	27	16
Correlation (r) with environmental factors			
Harvest date	0.404*	0.413*	0.048
Soil type	0.336*	0.184	0.118
Provenance	0.059	0.136	0.518*
Species	0.034	0.183	0.602*

differences were seen by provenance ($P = 0.030$). The foliar concentrations of Ca ($P = 0.0003$) and Mg ($P = 0.007$) also differed according to the seed origin, whereas that of Ni did not ($P = 0.585$).

Ratios between the total concentration of metal in seedling soil and that in leaves were used to estimate transfer or leaf-concentration factors. The soil-leaf transfer factors were correlated between the two soil treatments ($r = 0.97$, $P = 0.001$). For major plant nutrients (P, N, K and S), species typically have greater than unity transfer efficiencies (TEs). The two highest TEs were for Ca (10.63 0.88 for ultramafic; 7.57 0.56 for mafic) and N (4.43 0.44 for ultramafic; 5.31 0.54 for mafic), whereas the other major element of interest, Mg, indicated 0.35 0.39 for ultramafic and 1.23 0.16 for mafic soils.

The multi-way ANOVA (Table 6) clearly showed the dominance of the harvest-time effect in the TEs of major plant nutrients (N, P, K, S, Ca) as well as in those of Li, Ba, Mn and Cr. The seed-provenance effect was significant for a variety of trace elements and nutrients: Li, Na, S, Ca, Mn, Cr, Ni, Zn, Sr and Ba. As expected, soil type was a significant factor for nearly all of the nutrients and trace elements (exceptions were Na, Al and Fe). Most of the interactions were not significant, with the exception of the seed-provenance and soil-type interaction for the elements Li, S, Cr, Mn, Ni, Zn and Ba.

Seedling growth responses

Seedling mortality was low (3.75%), but telling. Eight of the nine plants that died were grown in ultramafic soil but had mafic provenance, and the remaining plant had ultramafic provenance and was grown in its home soil.

Significant differences in shoot (stem) height were evident for soil depth, type and species, although a variety of interactions were significant as well, clouding the interpretation (Table S1,

available as Supplementary material for this paper). Interaction plots were generated for each pair of factors recording significant effects (Fig. 3). The plot of provenance versus soil type suggested (although ANOVA $P = 0.18$) that seedlings whose acorns came from mafic soils tended to grow taller than those from ultramafic soils in mafic treatment; whereas seeds from mafic provenance grew less on the ultramafic treatment. Fig. 3b supports the conclusion that seedlings in the deeper mafic soils experienced a greater increase in shoot height. Graphs of species and soil type (Fig. 3c) and soil depth (Fig. 3d) generally show that seedlings grew taller in deeper soils and mafic soils, with differential results depending on species. Differences in growth on the basis of soil depth may also be driven by *Q. michauxii*.

As above, interaction plots were generated for those ANOVA interaction effects that were significant (Fig. 4). Figure 4a shows the RGR for soil type and soil depth, with mafic soil types outperforming their ultramafic counterparts at each soil depth. RGRs were high in the deeper soil treatments. Figure 4b details species RGR and soil type, with *Q. montana* showing the smallest differential among the soil types and *Q. michauxii* showing the biggest disparity in RGR among soil types, with *Q. alba* being comparable. Seedlings grown in deeper soils tended to have a higher RGR, with *Q. michauxii* showing the largest increase compared with other species (Fig. 4c). Interactions between seed provenance and species were examined; however, the wide standard error bars and lack of statistical significance preclude any meaningful interpretation (Table S2, available as Supplementary material for this paper).

Root to shoot data were investigated using a factorial ANOVA, and normality tests indicated that the model met ANOVA assumptions. Similar to the above data, interaction plots were generated for the significant interactions (Fig. 5; Table S3, available as Supplementary material for this paper). The root : shoot plot (Fig. 5a-c) combined with seed provenance and soil type indicated that seedlings with seeds taken from ultramafic sites and grown in the same soils had a higher root : shoot ratio ($P < 0.05$), and that those from mafic sites grown in the mafic soils did not show the same type of growth plasticity. Plants in ultramafic soil, regardless of provenance, had a greater root : shoot ratio, whereas Fig. 5d demonstrably shows that the root : shoot ratio of *Q. marilandica* as well as *Q. stellata* responded to the shallow soil treatment and both *Q. marilandica* and *Q. stellata* had higher root : shoot ratios than did the other species when all depths were averaged by soil type. Both *Q. marilandica* and *Q. stellata* had noticeably higher root : shoot ratios when resident seeds were grown in ultramafic soils.

For the three-way significant interactions, data were examined as subsets by species. Much of the data were inconclusive because of large standard error bars, but some information can be taken from Fig. 5. Root : shoot ratio varied by species and each species responded to treatments differently. An elevated root : shoot ratio was seen in serpentine soils for most species when grown in the shallow-depth treatment, other depths showed no significant differences or trends. *Q. michauxii* was the only species with a lower root : shoot ratio in serpentine soils, and this was the case for all depths. For some species, the soil type from which seeds were collected influenced root : shoot ratios and how the seedlings responded to the soil treatments. *Q. marilandica* and *Q. stellata*

Fig. 2. Foliar metal content for two oak species typically found as early colonisers of serpentine grasslands. Elements shown are standard diagnostic indicators of serpentine soil environments. There was only one independent analysis per treatment; therefore, no error bars are displayed. Typical instrument errors are $<1\%$.

Table 6. ANOVA results for leaf–soil transfer efficiencies as a function of harvest date, provenance and soil type

Data were calculated using extractable bases for sodium (Na), magnesium (Mg), aluminium (Al), calcium (Ca), manganese (Mn), iron (Fe) and zinc (Zn). Total metal values were used for the remaining elements. Significant ($P < 0.05$) differences are in bold

Factor	Element															
	Li	B	N	Na	Mg	Al	P	S	K	Ca	Cr	Mn	Fe	Ni	Zn	Ba
Harvest date	0.005	0.438	<0.001	0.011	0.508	0.071	<0.001	0.005	<0.001	<0.001	<0.001	<0.003	0.242	0.077	0.061	0.010
Provenance	<0.001	0.065	0.058	0.109	0.047	0.085	0.160	<0.001	0.812	<0.001	<0.001	0.304	0.162	<0.001	0.005	<0.001
Soil type	<0.001	0.071	0.052	0.032	<0.001	0.008	0.004	<0.001	<0.001	<0.001	<0.001	<0.046	0.001	<0.001	0.135	<0.001
Harvest date provenance	0.152	0.664	0.011	0.229	0.444	0.189	0.681	0.910	0.501	0.017	0.075	0.334	0.656	0.182	0.076	0.301
Harvest date soil	0.728	0.854	0.849	0.617	0.510	0.913	0.708	0.395	0.023	0.002	<0.001	0.086	0.251	0.103	0.604	0.384
Provenance soil	0.016	0.236	0.424	0.167	0.480	0.507	0.300	0.020	0.592	0.304	<0.001	0.481	0.439	<0.001	0.050	0.002
Harvest date provenance soil	0.289	0.979	0.758	0.977	0.683	0.992	0.389	0.801	0.747	0.051	0.176	0.362	0.680	0.159	0.904	0.502

Fig. 3. Interaction plots for mean shoot height, for provenance and soil type, soil type and soil depth, soil type and species, and soil depth and species. Shallow, 15 cm; medium, 30 cm; and deep, 60 cm. Interactions were all significant at $P = 0.05$. Error bars are s.e.

from serpentine soils had higher root : shoot ratios than did these same species from mafic soils. Additionally, seedlings from serpentine soils tended to have higher root : shoot ratios when grown in resident provenance soils. Data did not support this conclusion for seedlings from mafic soils.

Data for rooting depth are restricted to only the deeper (60 cm) growth tubes. Soil type and species main effects as well as their interaction effects were significant, with root depth being deeper in mafic soils for all five species (ANOVA, Table S4, available as Supplementary material for this paper). The provenance main effect had a P -value of 0.077, suggesting a possible trend. Figure 6 displays box-plot data for root depth by species and soil type. Clearly, seedlings grown in mafic soils had deeper roots than their counterparts grown in ultramafic soils. The degree

of difference between the soil types varied by species, with perhaps, the greatest relative difference occurring for *Q. alba*.

Table S5 (available as Supplementary material for this paper) documents the interaction effects and main effects associated with the mean number of leaves for each factor. Consistent with the other physiological data, plants generally had more leaves in deeper, mafic soils, and this varied by species (Figs 7, 8). *Q. michauxii* (mafic soils) had the highest mean number of leaves (14.1 $\pm$ 3.5) and *Q. marilandica* had the lowest (6.9 $\pm$ 2.2). Figure 7 shows the interaction plot between soil type and provenance for all species, suggesting that resident seedlings had more leaves than did non-resident seedlings.

Volumetric moisture content, collected for each seedling before gas exchange measurements, is presented in Table S6.

Fig. 4. Interaction plots for mean relative growth rate (mg g⁻¹ day⁻¹), for soil type and soil depth, soil type and species, and soil depth and species. Shallow, 15 cm; medium, 30 cm; and deep, 60 cm. Interactions were significant at $P < 0.05$. Error bars are s.e.

Soil moisture increased with soil depth in all treatments (see Table S6). Table S7 (available as Supplementary material for this paper) displays the results of a multi-way ANOVA, with both moisture content and stomatal conductance as dependent variables in response to the abiotic experimental treatments. Soil moisture and gas exchange displayed significant differences for all time measurements (with the exception of moisture in May for soil type). The only significant interaction was soil type by depth during two of the sampling periods (moisture in July and gas exchange in August).

The gas exchange and moisture measurements were examined using repeated-measures ANOVA (RMANOVA). The RMANOVA addresses how g_s and moisture differences change over time, whether there are any treatment by time

interactions, and whether time is a main effect for these data. Results of the RMANOVA are given in Table S8 (available as Supplementary material for this paper). Soil type, depth and species main effects were strong and significant with respect to differences in g_s , although provenance differences were not significant ($P=0.219$). Elevated values of g_s correlate to increased carbon fixation and the values were higher in the deeper ($P<0.001$) and mafic ($P<0.001$) soils. The within-subject error component of the RMANOVA showed that there was a significant time effect on gas exchange ($P<0.001$). Significant two-way interactions occurred between time and soil depth, type and species ($P<0.001$). All seedlings showed higher conductance in mafic and deeper soils. *Q. marilandica* and *Q. stellata* consistently maintained high gas exchange rates during all sampling periods, with *Q. alba*, *Q. montana* and *Q. michauxii* showing lower rates, respectively, in that order (Figs 9, 10). The two-way interaction between time and provenance was not significant ($P=0.086$). No three-way interactions were significant and, as such, are not included in Table S8, for simplicity.

Discussion

Mixed oak forests sustain a diverse flora and fauna, and are highly valued for their conservation and economic attributes throughout eastern North America. However these forests have changed dramatically during the past two centuries (Dyer 2001; Abrams 2003; O'Dell *et al.* 2006; McEwan *et al.* 2011). Traditional mid-Atlantic serpentinite terranes also harbour unique mixed oak savannas, which have also declined markedly during the 20th and 21st centuries (Tyndall 1992). The oak species in the present study can be found on a wide variety of substrates and climatic conditions, and, accordingly, one might expect these oaks to display evidence of local adaptation to an environmentally heterogeneous landscape.

We examined how patterns of tree seedling mortality, growth, biomass allocation, leaf gas exchange, and leaf metal and nutritional content vary along experimental gradients of soil depth and moisture under two soil types. The experiment was conducted on seedlings growing for less than 1 year, and, as such, does not assess long-term survivorship or other larger ecological problems and may have limited microbial community differentiation. The soil types are similar with respect to common nutrients (N, P, K), but are chemically imbalanced with respect to Ca:Mg ratios, Cr and Ni content. Additionally, soils collected at the ultramafic site are shallow (<10 cm). When these facts are taken together, they create an edaphic system that is harsh for plants. Consistent with Abrahamson (1979), although not statistically significant, seeds for four of the five of those *Quercus* species collected on the ultramafic sites produced a smaller seed size than did the same species seed mass in more optimal sites (mafic), but only two of these four (*Q. montana* and *Q. alba*) were statistically significant.

One might expect edaphic factors to play a fundamental role in bacterial and fungal physiology and ecology and also affect seedling performance. Soils yielded slightly higher fungal content in the ultramafic soils and no evidence of mycorrhizal colonisation in either soil type, which was likely to be due to the breakage of mycorrhizal associations during homogenisation.

Fig. 5. Interaction plots for root to shoot ratio means, for provenance and soil type, soil type and species, soil depth and species, and provenance and species. The diagram showing soil depth, type and root to shoot mean ratios, only uses data from the 15 cm depth treatment. Medium and deep treatments displayed no differences. Interactions were significant at $P = 0.05$.

Fig. 6. Rooting depth obtained at the end of the growing season harvest for each species and soil-type treatment. Error bars are s.e.

Intense homogenisation is akin to agricultural tillage that results in fungal : bacterial ratios of <1 (Hendrix *et al.* 1986). Both soil types displayed fungal : bacterial ratios of <1 , which suggests that fungal communities did not play a significant role in seedling performance. Grassland soils, such as those used in the present study, and early successional soils are typically bacterially dominated (Ingham *et al.* 1989). Mature forest soils typically have total bacterial to total fungal biomass ratios significantly greater than unity (Ingham *et al.* 1989). Many researchers have documented that fungi are more acid tolerant than bacteria, leading to increased fungal dominance in acidic soils (Högberg *et al.* 2007; Joergensen and Wichern 2008; Rousk

Fig. 7. Interaction plot that details the total leaf means, separated by provenance and soil type. Interactions were significant at $P = 0.05$. Error bars are s.e.

et al. 2009). The experimental soils were biologically neutral, perhaps partly explaining the bacterial dominance in both experimental soils.

Oak seedlings in the present study were subjected to varying levels of stress associated with soil nutritional imbalances (Ca : Mg), combined with shallow soils and concomitant lower moisture levels. Most oaks in North America have the ability to adapt to water stress through avoiding or tolerating drought (Abrams 1990). Some adaptations to drought stress are thick leaves, small stomata (Abrams 1990) and deep-penetrating root

systems (Abrams 1990; Pallardy and Rhoads 1993). Increased stomatal conductance can help reduce leaf temperatures but with the trade-off of desiccation and cavitation. However, such

tolerance and traits may differ considerably among oak species and provenances, reflecting adaptation to environments with different water availability (Dickson and Tomlinson 1996) and soil fertility (Anacker *et al.* 2011). Edaphic stresses where woody species are exposed to high levels of toxic metals may display reduction in photosynthesis, water uptake and nutrient uptake, and may show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and mortality (Yadav 2010).

Foliar content

The different soil types and chemistries affected the physiological growth and leaf chemistry of the oak seedlings. Soil pH heavily influences plant elemental uptake, yet these two soils are both effectively neutral because of the high base cation content. The neutral pH, combined with the lack of mycorrhizal association, may have limited metal uptake. However, there are significant differences in chemistry that were, in part, reflected in leaf concentration.

The following leaf foliar responses were observed: (i) nutrient concentrations (P, N, K, S) were similar regardless of soil

Fig. 8. Interaction plot detailing the mean number of leaves, separated by species and soil type.

Fig. 9. Interspecific variation in mean stomatal conductance for each species over the four sampling periods. Interaction plot shows experimental tree species and mean stomatal conductance over time. Error bars are s.e. Interactions were significant at $P = 0.05$.

Fig. 10. Interspecific variation in mean stomatal conductance from the August dataset, separated by soil depth. Error bars are s.e.

treatment and were significantly correlated with harvest time, (ii) Ca, Mg, Mn, Ni and Zn are strong discriminators of the data and are shown to be sensitive to soil type and seed provenance. Considering that the ultramafic soils are generally quite depauperate in Ca, elevated leaf Ca:Mg ratios relative to the extremely low Ca:Mg ratios in the serpentine soils suggest selective Ca uptake and that the more mesic of these species have an increased metabolic need for Ca, whereas *Q. stellata* and *Q. marilandica* can grow in more Ca-poor areas, such as serpentine terrains. This translocation to the leaves and the inhibited Mg translocation and subsequent sequestration by roots, may be important adaptations for these species (O'Dell and Rajakaruna 2011). O'Dell *et al.* (2006) noted similar adaptations in Californian serpentine species, Mn appears readily transferred from soils to leaves, which is not surprising, considering that it is a physiologically active component for photosynthesis (Römhild and Marschner 1991). Similar chemistry on several serpentine oaks shows that Mn is readily translocated from roots to shoot (O'Dell *et al.* 2006). Accumulations of Ni may have the evolutionary advantage of protection against herbivores as well as allelopathy (Boyd and Martens 1998; Kazakou *et al.* 2008). Chromium, a signature element in ultramafic soils, has transfer efficiencies considerably less than unity, indicating active exclusion mechanisms and (or) uptake, consistent with the very low solubility or bioavailability of Cr in these soils (Vithanage *et al.* 2014).

Many elements that displayed significant differences in the statistical analysis, such as Li and boron (B), even though plants take them up, give no indication of being major players in serpentine environments. For example, Li appears not to be required for plant growth and development. However, this question is not yet completely resolved, because, in the $\mu\text{g kg}^{-1}$ range, stimulatory effects of Li on plant growth have been observed (Hawrylak-Nowak *et al.* 2012). Boron and other elements are required for plant growth to give structure and functionality to certain biomolecules; however, they are necessary only in minute quantities (Warington 1923; Gupta and Solanki 2013).

The relative similarity in transfer efficiencies and elemental accumulations between nutrient-rich mafic soils and the low productivity of ultramafic soils suggests that these oak species have adaptations designed to reduce nutrient losses and optimise nutrient-use efficiency in diverse settings, including in harsh serpentine areas.

Seedling growth

The following general growth responses were observed: (1) seedlings derived from plants growing in serpentine soils had higher survival than seedlings derived from plants growing in mafic habitats, when both sets of seedlings were grown in serpentine soils, (2) shoot growth was reduced in shallow soils and serpentine soils, (3) RGR was species specific, but generally enhanced in mafic and deep soils, (4) *Q. michauxii* and *Q. montana*, respectively, had the highest and lowest relative growth rates of the species, (5) *Q. marilandica* and *Q. stellata* shoot height, relative growth rate and root:shoot ratio main effects and interactions with soil type are suggestive of local adaptation to soils, (6) elevated root:shoot ratios were apparent in serpentine and shallow soils, (7) root elongation was more

prevalent in mafic soils, and (8) mean leaves per plant suggest that resident seedlings grown in their native or local soils have higher growth rates.

Each species exhibited unique physiological responses to the soil resource base, but some trends are apparent. The differential mortality between seedlings derived from ultramafic systems (6.7%) and those from mafic soil systems (0.8%), when both were planted in ultramafic soils, suggests a local ecotypic effect. Edaphic properties (physical or chemical) were the likely drivers of any such effect. *Quercus marilandica* and *Q. stellata* seedlings allocated more carbon to root systems, whereas RGRs and stem heights were lower than those for *Q. michauxii*, equal to those for *Q. alba* and greater than those for *Q. montana*. This allocation to storage and slow growth rate is an effective strategy of drought tolerance and can mediate the impacts of other stressors such as nutrient deficiency (Abrams 1990) and is consistent with trait-mediated systems (Cornwell and Ackerly 2009). Oaks generally allocate more carbon to roots than do other tree species in the region. For instance, from greenhouse data, the root:shoot ratio of seedlings of *Liriodendron tulipifera* L. (tulip poplar) is 0.37 (Kolb *et al.* 1990; Loats and Rebbeck 1999), *Acer rubrum* L. (red maple) 0.39, and *Fagus grandifolia* Ehrh (American beech) 0.52 (Loach 1970). In the present study, root:shoot ratios of all oak seedlings were greater than one, with *Q. stellata* and *Q. marilandica* having the highest ratios of 9.5 and 13, respectively. Allocating more C to the root system helps maintain a positive water balance during dry periods and ensures the plants are well adapted to shallow soils. Of course, maternal effects can influence the results of local-adaptation studies; however, with the long-lived oaks, time constraints on experimental duration do not typically allow for direct fitness examination of offspring (Mousseau and Fox 1998; Wright and Stanton 2011; Bieger *et al.* 2014).

Shallow soils had the general effect of eliciting higher root:shoot ratios and lower RGRs. Such soils would further constrain root development with concomitant loss of fixed carbon. Shallow soil usually limits water-holding capacity, intensifying environmental filtering. Thus, the more xeric-oriented species would require a higher root:shoot allocation to mitigate the inherent low moisture content of shallow serpentine soils. Moisture stored at depth would favour deep-rooted woody plants (Walter and Mueller-Dombois 1971; Walker and Noy-Meir 1982), but such water storage is not possible in serpentine areas with extremely shallow soil profiles underlain by bedrock.

Stomatal conductance

Stomatal conductance is a measure of gas exchange reflecting how many stomata, and to what extent, are open. Plants that undergo drought stress often have a decreased g_s compared with plants not exposed to drought stress (Balok and Hilaire 2002). Figure 10 details how seedlings in this experiment consistently have lower conductance for all species in the shallow soil treatments, which also had the lowest moisture content. The following summarises the gas exchange response: (1) gas exchange was significantly higher for seedlings in nutrient-rich mafic soils and deeper soils; (2) conductance changed dramatically over the sampling period, with the lowest g_s values during the warmer drier late summer period; and (3)

species responded consistently, although differently, over time, with *Q. marilandica* and *Q. stellata* consistently exhibiting higher gas exchange rates. Many plants from xeric provenances have the ability to transpire and survive even when soil moisture is low. The higher g_s values indicate that the plant is under less stress; however, water leaves the leaf through the stomata, so greater g_s would increase water loss. Although seemingly a paradox, a greater g_s allows plants the ability to cool themselves and maintain a lower leaf temperature, thereby limiting losses caused by transpiration.

Conclusions

Results from this reciprocal transplant study showed that initial seedling growth of five common upland oak species from eastern USA can be considerably different. The oak species responded differently to changes in soil depth, nutrient content (soil type) and seed environmental origin. Transfer efficiencies for the elements from soil to leaf are highly correlated across species and soil treatments, with perhaps minor deviations for the elements Ca, Mg and Ni for the serpentine savanna species. *Quercus marilandica* and *Q. stellata* displayed significantly higher efficiencies for the critical element Ca. Seedlings, regardless of seed origin, soil depth or species displayed more robust growth in mafic than in ultramafic soils. Soil depth was also an important determinant of increased plant growth, with all species having greater growth in the deeper-soil treatments. Accordingly, the present study suggests the following ranking of species from more xeric-adapted to less xeric-adapted: *Q. marilandica* > *Q. stellata* > *Q. montana* > *Q. alba* > *Q. michauxii*.

Stomatal conductance indicates that seedlings were more stressed in shallow and serpentine soils, with a clear species hierarchy for maintaining gas exchange during dry conditions. Evidence from interaction effects concerning the stomatal conductance, number of leaves and shoot height (both proxies for plant fitness) supported the hypothesis of local adaptation, at least at the seedling stage. These data have implications for serpentine-savanna restoration efforts and for managing deciduous mixed oak forests. Seedlings for planting should be derived from a local source with similar ecophysical settings. Additionally, these data indicated that as serpentine savanna soils mature and thicken in response to woody encroachment, the more mesic oaks, *Q. alba*, *Q. montana* and *Q. michauxii*, might become competitively superior to slower-growing *Q. marilandica*.

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Conflicts of interest

No conflicts of interest.

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