Neoformation of clay in lateral root catchments of mallee eucalypts: a chemical perspective

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Background and Aims A previous paper (Annals of Botany 103: 673–685) described formation of clayey pavements in lateral root catchments of eucalypts colonizing a recently formed sand dune in south-west Western Australia. Here chemical and morphological aspects of their formation at the site are studied.

Methods Chemical and physical examinations of soil cores through pavements and sand under adjacent heath assessed build-up of salts, clay and pH changes in or below pavements. Relationships of root morphology to clay deposition were examined and deposits subjected to scanning electron microscopy and energy-dispersive X-ray analysis. Xylem transport of mineral elements in eucalypt and non-eucalypt species was studied by analysis of xylem (tracheal) sap from lateral roots.

Key Results The columns of which pavements are composed develop exclusively on lower-tier lateral roots. Such sites show intimate associations of fine roots, fungal filaments, microbiota and clay deposits rich in Si, Al and Fe. Time scales for construction of pavements by eucalypts were assessed. Cores through columns of pavemented profiles showed gross elevations of bulk density, Al, Fe and Si in columns and related increases in pH, Mg and Ca status in lower profiles. A cutting through the dune exhibited pronounced alkalinity (pH 7–10) under mallee woodland versus acidity (pH 5–6.5) under proteaceous heath. Xylem sap analyses showed unusually high concentrations of Al, Fe, Mg and Si in dry-season samples from column-bearing roots.

Conclusions Deposition of Al–Fe–Si-rich clay is pivotal to pavement construction by eucalypts and leads to profound chemical and physical changes in relevant soil profiles. Microbial associates of roots are likely to be involved in clay genesis, with parent eucalypts supplying the required key mineral elements and carbon sources. Acquisition of the Al and Fe incorporated into clay derives principally from hydraulic uplift from ground water via deeply penetrating tap roots.

Key words: Niche construction, eucalypts, root morphology, xylem transport, hydraulic lift, element mining, soil formation, biomineralization, soil pans, duplex soils.

INTRODUCTION

A recent study (Pate and Verboom, 2009) presented evidence of extensive clay pavements in the lateral root catchments of eucalypts colonizing a lunette-type dune of quartzitic sand blown out of a playa lake during late Pleistocene times. Morphologies of pavements were described and the closely spaced columns of which they are composed were suggested to have been constructed by the eucalypts from locally sourced and imported constituents. The same pavements were held responsible for marked reductions in density and extents of build up of salts and clay components in and around developing columns. Chemical analysis of soil cores passing through currently forming columns are compared with those through adjacent unmodified sand to assess respective increases in bulk density and extents of build up of sands and clay components in and around developing columns. Material from sites of nascent column formation are subjected to scanning electron microscopy and targeted micro-analysis to describe the morphology, mineralogy and chemical and biotic properties of the clay-forming process.

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Data from seasonal collections and analyses of xylem sap from eucalypt and non-eucalypt species test the hypothesis that hydraulic uplift and lateral redistribution of xylem-borne mineral elements acquired by tap roots from lacustrine deposits below the dune may deliver the bulk quantities of certain key mineral elements necessary for clay pavement construction.

A major aim of the paper is to quantify rates and amounts of clay synthesis in pavements laid down by mallee woodland at the dune. Two sources of data are analysed. One of these involved assessments of weights of partly completed pavement laid down by a single study tree sited at the front of eucalypt colonization near the crest of the dune. The other comprised sampling of fully formed mature pavements laid down by generations of eucalypt woodland on mid- and lower regions of the lake-facing slope of the dune. This information is then viewed in the context of the proposed mechanisms and chemical processes studied elsewhere in the paper.

METHODS AND MATERIALS

Study site and rooting morphology and pavement formation under study species

The lunette at which the study was conducted is sited within the Lake Chillinup Nature Reserve (grid ref. 34°32′54″S, 118°04′12″E) in the South Stirlings Region of Western Australia. Digital elevation models of the area, maps of distribution of mallee woodland and heath and associated soil profile characteristics have been already described by Pate and Verboom (2009).

The principal study species was the mallee E. incassata Labill. (see Brooker and Kleinig, 1990), and detailed examinations of its root morphology and pavement formation were conducted on a single tree near the dune crest where mallee woodland abutted on myrtaceous:proteaceous heath. Using a combination of pit excavations and air spading, spatial relationships were investigated between roots, mature clay columns and columns currently under construction. Small-sized precipitations of clay in and around clusters of fine roots were identified as foci of column formation and their fine surface detail revealed by low energy spray-irrigation sufficient to remove sand grains surrounding the column while not disrupting fine roots and associated clay.

Fine structure of nascent and mature columns

Material harvested from the apical region of currently forming and previously formed clay columns was subjected to microscopic, chemical and mineralogical examination using a Phillips (FEI) XL40 scanning electron microscope operated under controlled pressure and coupled to an energy dispersive X-ray analysis (EDX) unit. Samples comprising fine roots and associated clay-containing rooting substrate were carefully removed with tweezers and mounted on glass slides. Material was first observed uncoated and then after coating with gold. Low-power observations on the uncoated samples were conducted using a chamber pressure of 0.5–0.8 mbar, an operating voltage of 30 kV and a Robinson back-scatter detector to identify chemically and mineralogically distinct particles in the column material. Samples coated with gold were employed to view material at high resolution (vacuum of 1 × 10⁻⁶ mbar and operating voltage of 15 kV). EDX analysis on selected features in coated or uncoated specimens, used spot sizes of 1–2 μm and counting times of 45–60 s.

Relationships between pavement production and tree biomass

Probings by hand of the lateral root catchment of the study tree using a stainless-steel rod (see Pate and Verboom, 2009) demonstrated that closely adjacent clay columns extended radially for up to 3.5 m outwards from the lignotuber. Selecting three opposing radii within the rooting catchment, hand-excavated inspection pits 1.5 m wide were dug along each to a depth of 2 m, namely to at least 75 cm below the base of the lowest clay columns. Each pit extended 6 m out from the lignotuber to 2.5 m beyond the last identifiable columns. Exhaustive collections of column material were then undertaken for successive series of 50-cm sections of pit face taken along the whole of each radius. Each 50-cm section was excavated 20 cm horizontally into the pit face, i.e. a sample slab of 0.2 m² (50 × 20 × 200 cm). With say ten such slabs taken from the face of a pit, the combined profile material would have effectively sampled 1 m² of root catchment surface area and in the process effectively recovered all underlying column material.

After examining rooting morphologies and sub-sampling of column material as described above, trunks, leafy canopy, live lignotuber and proximal regions of tap roots were harvested. All column and plant material was then dried and weighed. Separate assessments were also made of the number and mass of currently forming columns associated with each R2 root uncovered during the respective excavations.

Chemical composition of soil profiles in the reserve under myrtaceous:proteaceous heath and eucalyptus woodland

Elemental composition of profiles in lateral root catchments of E. incassata, E. pleurocarpa and E. occidentalis were assessed by 3-m-deep coring at sites carefully selected on the basis of prior rod probings to pass directly through columns. Maturing columns under the study tree of E. incassata were cored in triplicate to match a similar set of corings through unmodified (unpavedment) sand under closely adjacent myrtaceous:proteaceous heath.

Samples from a series of depth intervals down each core were oven dried and coarse matter (principally root material) removed with a 2-mm sieve. Appropriately sized sub-samples were fused with sodium peroxide and the melt dissolved in dilute (10 %) HCl. Subsequent chemical analyses involved determinations of total Fe, Al, Ca, K and Mg using a Perkin-Elmer (Optima 3000) inductively coupled plasma (ICP) optical emission spectrometer (OES). Thorium (Th) levels were determined using a Perkin-Elmer (Elan 6000) ICP mass spectrometer after the melt had been dissolved in 2 % HNO₃. Appropriate standards and duplicated analyses of aliquots of selected samples were included in each run. Additional analyses of core material were conducted using procedures and methodologies set out by Rayment and...
Higginson (1992). These included: electrical conductivity and pH of aqueous extracts using soil:solution ratios of 1:5 (Method 4A1); assays of exchangeable cations Ca, Mg, Na, K extracted using 0.1 M BaCl2/0.1 M NH4Cl (Method 15E1) and measurements of oxalate-extractable Al, Fe and Si using an ICP:OES after 1 part of soil had been tumbled with 33 parts of Tamm’s reagent for 1 h (Method 13A1). Values for % carbonate were based on pressure increases in a closed vessel following neutralization of the sample with HCl–FeCl2 solution (Method 19B1).

Comparisons of Fe, Al and Si concentrations in leafy shoots of study eucalypts and other shrub species on the dune

Leafy shoot material was collected from 25 widely separated plants of each of the three mallee eucalypts and of five principal non-eucalypt shrub species. Material was dried at 60 °C, ground and acid digested prior to analysis for the three elements by ICP:OES as described above.

Mapping of pH changes across a cutting through the dune

The principal features of the 200-m-long man-made cutting through the dune have already been described by Pate and Verboom (2009) in relation to pavement morphologies and visual evidence of mineralization under mallee woodland, versus the mild podzolic characteristics and absence of substantial mineralization under nonpavemented myrtaceous proteaceous heath.

Selecting a series of sampling stations along the length of the cutting, pH of soil was assessed at successive depths below each station. Trends in pH across the face of the cutting were then described using ordinary Kriging (see Myers, 1991) implemented in GeoMedia Grid (Version 6.0). This depiction of pH was then distorted by Intergraph MGE Projection Manager (Version 8.0) to match a fisheye photographic view available for the cutting.

Physical composition of soil profiles

Particle size distributions, conducted on the same soil samples collected for chemical analyses, were accomplished using stacked 1, 0.6, 0.3, 0.18, 0.15, 0.106 and 0.075-mm-diameter sieves and the pipette method of Indorante et al. (1990). Samples were pre-treated with hydrogen peroxide to remove organic matter, and 1:1 Calgon–sodium hydroxide mixture to disperse particles. Coefficients of uniformity (CU) were estimated as d60(mm)/d10(mm), where d60 and d10 represent the hypothetical sieve diameters in millimetres, through which 60% and 10% by weight of the sample would pass, respectively. Relevant d60 and d10 values were interpolated from plots of cumulative weight percentage versus d, with the latter defined as $-\log_{2} d$ where d = diameter of retaining sieve in millimetres.

Samples taken in triplicate from profiles under native vegetation in the Reserve were assessed for bulk density using techniques described by Blake and Hartge (1986). Where soil was loose, a bevelled steel core (70 mm long and 73 mm in diameter) was driven into a clean vertical face and the resulting cored material dried, weighed and its bulk density determined by relating core weight to volume. Where the soil was brittle and hard, a wax technique was employed involving coating of a clod of known dry weight with paraffin wax and applying Archimedes’ Principle to assess volume.

Xylem sap collection and analysis

Xylem (tracheal) sap was obtained by vacuum extraction of clean, bark-free xylem cores cut from freshly excavated 30- to 40-cm-long sections of healthy lateral root. The technique was essentially as described and illustrated by Pate et al. (1994). Attempts were made to collect sap on a monthly basis, including randomly selected night- and day-time occasions. Samples related mostly to the principal study species E. incrassata, but sap was also collected from three other eucalypts (the mallees E. pleurocarpa and E. decipiens and the tree species E. occidentalis). A selection of large shrub or tree species of Proteaceae were also used for xylem sap collection. All samples were frozen immediately after collection, unfrozen prior to analyses, and then passed through Pall Gelman (Ann Arbor, MI, USA)/GH Polypro membrane filters to remove particulate matter. Analyses for Al, Fe, Ca, Mg, Na, K, and Si were conducted on sap samples diluted with 10% HCl, using an ICP:OES and the quality control procedures described above. Organic acids in sap were analysed by reversed-phase column liquid chromatography (see Cawthray, 2003).

RESULTS

Morphology, rooting architecture and clay pavements of the mallee E. incrassata

The developmental morphology of early growth of seedlings and saplings of the species is basically as described by Wildy and Pate (2002) for the oil mallee Eucalyptus kochii. Each seedling quickly develops a stout, deeply penetrating tap root on the upper flanks of which a number of laterals radiate outwards into the surrounding soil. When the leafy stem is 30 cm high and the tree probably no more than 10 years old, a noticeably swollen lignotuber of up to 1.5 cm diameter will have typically formed at the junction of shoot and root. Excavations of older juvenile trees ≥1 m high show that their primary tap roots have penetrated to at least 2 m depth and larger laterals have extended radially outwards for 1–2 m beyond the projected area of the canopy. There is no evidence of clay pavement formation at this stage.

It was estimated that the majority of trees of E. incrassata at Chillinup were 200–300 years of age. Many showed central decay of their lignotubers, presumably following fire or attack by termites. Judging from the presence of large areas of double pavement across certain parts of dune, the current mature woodland occupying such regions probably represents a second phase of colonization by eucalypts. Juvenile species are now scarce throughout the native vegetation at the site, although recruiting is prolific along adjacent roadside verges.

The mature tree selected for study is depicted stylistically in Fig. 1. Aged at least 150 years, it currently bore two living trunks shown by ring counts to be of 23 and 57 years age. Each trunk was attached proximally to live lignotuber tissue.
(LLt) which comprised a flange-like ring surrounding the long-decayed primary lignotuberous stock (DLt). Stumps of earlier-formed shoots and remains of the primary taproot system (DPTr) were attached to dead central parts of the lignotuber. Two live secondary tap roots (STr) of similar vintage to the live trunk descended from the lignotuber and the upper parts of these and adjacent flanks of live lignotuber bore lateral roots (see Fig. 1). The tree currently possessed 16 live laterals. Selected ones of these were subject to careful spade-based excavations to assess total length, depths, branching patterns and association or otherwise with new columns currently being added to the already extensive but as yet incomplete clay pavement constructed by the tree.

One class of lateral (R1, Fig. 1) was borne on the upper flanks of the lignotuber and extended outwards for 8–12 m within the depth zone 10–30 cm. The seven roots of this upper tier showed little major branching, attenuated in diameter only slightly with distance from the bole, and carried dense clothings of fine rootlets. The latter were profusely mycorrhizal during the wet season and accordingly presumed to function as major sites of uptake of water and nutrients from upper soil horizons.

The second class of lateral roots (R2, Fig. 1), comprised a lower tier arising from basal regions of the lignotuber or topmost regions of a tap root. Lateral of this type extended for up to 12 m and remained strictly within a depth zone 30–80 cm. The R2 roots which reached furthest developed side branches (SB, Fig. 1) borne at approx. 70-cm spacings along the root length. Branched and un-branched R2 roots mostly carried vertically descending mini-sinker roots (MSR) and associated newly forming columns (NC). Where several developing columns were associated with a single mini-sinker, the size and presumed age of the cohort decreased distally (see Fig. 2A–D). Other stouter and more deeply penetrating sinkers (SR) were also borne on some R2 roots. These did not form columns and were presumed to exchange water between the tree and...
lower regions of the profile. However, judging from their attenuation in diameter, they were unlikely to penetrate much more than 2 m.

Most large columns of the pavement of the study tree were not associated with live R2 roots and since carbonized remains of R2-type roots were often found in close vicinity (see designations in Figs 1 and 2A), they were likely to have been constructed during an earlier phase of tree growth.

Based on evidence obtained from the large number of roots excavated at various times for xylem sap analysis, the partner mallees *E. pleurocarpa* and *E. decipiens* and the tree eucalypt *E. occidentalis* also developed R1- and R2-type roots which, respectively, showed similar characteristics in terms of mycorrhization and sittings of pavement construction as described above for *E. incrassata*. The reader is referred to Pate and Verboom (2009) for more general descriptions of pavement morphologies of the eucalypts and their effects on understorey vegetation at Chillinup.

**Microscopic structure of forming columns**

The scanning electron micrographs (Fig. 3A–E) of gold-coated specimens of material obtained from the topmost region of an embryonic column reveal complex spatial relationships between deposited clay, siliclastic matrix and biota. Figure 3A, B and E shows interwoven mycelial structures (MF) overlying root surfaces. Clayey precipitates (CD) appear to have flooded and coated root, fungal and sand grain components and crystalized out as kaolinite plates. However, it is possible that nano-sized regions of allophane and immogolite may be present and further mineralogical study is required to establish the true nature of these mineral assemblages.

There is no evidence of mycorrhizal association between root and fungal components. A segmented bacterial colony is seen in Fig. 3A and B while Fig. 3C shows a root surface close to sites of clay genesis, with a root hair (R) borne on the same root. The terminal portion of the hair is enlarged in Fig. 3D.

Figure 3F represents a low-power electron backscatter image derived from an uncoated portion of previously formed column. Uniformly sized sand grains (SG) of the original dune deposit are visible alongside senesced mycelial filaments, with both components carrying parts of their original clay coatings.

Figure 4 presents data for electron microprobe analyses of the 1-μm-diameter target areas designated 1, 2 and 3 in the scanning electron microscope scans of Fig. 3. Clayey precipitates analysed at target area 1 (Fig. 3B) are seen to be particularly concentrated in Al and Si while also containing detectable amounts of K, Fe and possibly Ca and Mg. Proportional signal sizes for the above elements are more or less in accordance with the extents of incorporation recorded for chemical analyses of forming columns (see next section). The EDX microanalysis of surface deposits on the root hair surface (target area 2, Fig. 3D) also shows intense precipitation of aluminosilicates and Fe compounds together with lesser amounts of K, Ca, Mg and Ti. Analysis of previously formed column material (target area 3, Fig. 3F) shows particularly high levels of Si relative to Al and Fe and the presence of minor components K, Mg, Ca and Ti. Small amounts of S and Cl are also detected at target area 3. Presence of these would have been masked by the Au peak in EDX spectrographs of the gold-coated preparations (target areas 1 and 2, Fig. 4).

Bulk mineralogical identifications using X-ray diffraction (M. Aspandiar, unpubl. res.) taken together with the analyses mentioned above would indicate that nascent columns are mostly composed of poorly ordered silicates and kaolinite alongside minor hematite and goethite components.

It should be pointed out that the microprobe data (recorded in Fig. 4, 1 and 2) relate to column material originally containing live biotic components and that these were desiccated during specimen preparation. Artefacts would therefore be expected from leakage and capillary movement of solutes from ruptured roots, mycelial filaments and microbes. However, while probably prominent in relation to major tissue constituents such as K, Ca and Mg, such artefacts of specimen preparation would probably be of much lesser significance in relation to the largely insoluble Al-, Si- and Fe-based components of the deposited clays under examination. A further issue is that mild pressure was inevitably applied during specimen collection and preparation and this, coupled to dehydration of previously hydrated components, would inevitably lead to displacement and distortions of particles, albeit minimal in respect of clay coatings.

**Relationships between pavement production and tree biomass**

Dry weights of column material, collected radially outwards along each pit face, were used to assess total weight of columns in the rooting catchment of the study tree. Transformation of data required corrections for progressive area increases of successive annuli out from the lignotuber. Total weight of column material in the 40-m² root catchment of the study tree was then found to amount to 2900 kg dry weight, or the equivalent of an average deposition of 75 kg across each square metre of catchment.

The above-ground biomass of the parent tree comprised 75 kg of leafy trunk material and 37 kg of live lignotuber. It was impossible to collect all root biomass so we had to rely on estimates of biomass distribution between shoot, lignotuber and roots provided by Wildy and Pate (2002) for 4-year-old trees of the oil mallee *Eucalyptus kochii*. Their study gave a dry weight ratio of leafy trunk plus lignotuber to roots of 0.9, and, assuming that similar allometric relations applied to *E. incrassata*, total live biomass of the study tree would then be 237 kg. The three R2 roots whose complements of forming columns were exhaustively excavated, harvested and weighed separately contained an average of 9 kg of column material per root.

**Chemical and physical composition of soil profiles in the reserve**

Figure 5 provides data for total amounts of Al, Fe, Th and oxalate-extractable Al, Fe and Si in profiles passing through nascent columns of *E. incrassata* and compares these values with comparable data for sand under adjacent heath. Sites of column formation are seen to have intensively accumulated all of the listed elements and their extractable analogues. However, with the exception of Si located in alkaline
**FIG. 3** Scanning electron micrographs of gold-coated specimen from the apex of a nascent column (A–E) and uncoated specimen of earlier-formed column (F): (A) specimen showing clay deposits and coatings (CD) on root surface (R) and network of mycelial filaments (MF); (B) high-power magnification of lower right region of (A) demonstrating segmented bacterial colony (BC) among clay-coated mycelial filaments; (C and D) low- and high-magnification views of root hair borne on column-forming root; (E) amorphous and crystalline (indicated by an asterisk) clay deposits on and amongst mycelial filaments; (F) low-power backscatter view of earlier-formed column showing lightly coloured fragmented remains of mycelial network amongst duller sand grains (SG) of the original dune matrix. Brightness of the clay deposits overlying mycelial filaments signals the presence of heavy elements (probably Fe; see analysis in Fig. 4-3). Areas labelled 1, 2 and 3 in the figure refer to targets subjected to the EDX analyses illustrated in Fig. 4.

**FIG. 4** Electron probe analyses of elemental composition of clayey precipitates on target areas 1, 2 and 3 indicated in Fig. 3: (1) clay coating on root surface of nascent column with major peaks for Al and Si and minor peaks for Fe and K; (2) clay coating on root hair from nascent column showing intense Si, Al and Fe peaks with lesser ones for K, Ca, Mg and Ti; (3) earlier-formed column showing dominant peak for Si, major peaks for Al and Fe and lesser signals from K, Mg, Ca and Ti. Signals for Au in (1) and (2) derive from gold coating.
regions immediately below pavements (W. H. Verboom, unpubl. res.), there were no signs of significant depletion (mining) immediately above or below the forming columns. Inclusion of data for Th is justified because of its significance as a radiometric marker of neoformation phenomena. Exemplifying an element lacking redox chemistry and essentially absent from the dune matrix, its transfer and build up within the plant–soil environment is highly likely to be mediated by chelation processes (see Verboom and Pate, 2003).

Table 1 summarizes information from a large body of information (primary data not presented) on key chemical and physical characteristics of cores passing through mature columns under *E. incrassata*, *E. pleurocarpa* and *E. occidentalis* and through forming columns of the study tree *E. incrassata*. The material included comparable data for cores through the weakly podzolized sand typical across heath adjacent to the mallee woodland at the site (see vegetation map given in Pate and Verboom, 2009).

Conclusions to be drawn from Table 1 are as follows. (a) Levels of amorphous (oxalate-extractable) Al, Fe and Si are highest in zones where columns are actively forming (Table 1A). (b) Biogenic clay, and to a lesser extent carbonates (see entries in Table 1A), are at highest levels below mature columns in profiles with a protracted history of eucalypt occupation. It therefore seems that mineralization of lower profiles must continue long after initial invasion by mallees. (c) Exchangeable Ca : Mg ratios are greater than unity in the podsol, but drop markedly below 1 in zones high in precipitated clay (last column in Table 1A). (d) Increases in soil bulk density

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**Fig. 5** Chemical composition of soil cores (triangles) passing through forming columns of *E. incrassata* compared with that through matching sand (squares) under adjacent heath. Elements selected for total analysis are Al, Fe and Th with oxalate-extractable counterparts shown for Al and Fe. Levels of amorphous Si, indicative of biological processing of the element are also illustrated. All values given are on a mass basis. Note that oxalate digestions would dissolve imogolite-type materials but very little biogenic opal and would thus exclude phytoliths.
### Table 1. Selected characteristics of soil profiles under myrtaceous:proteaceous heath versus eucalypt woodland

#### (A) Chemical and physical properties of comparable profile horizons with or without column material

<table>
<thead>
<tr>
<th>Horizon*</th>
<th>Clay (%)</th>
<th>Bulk density (mg m⁻³)</th>
<th>Total Al (g kg⁻¹)</th>
<th>Total Fe (g kg⁻¹)</th>
<th>Oxalate Al (mg kg⁻¹)</th>
<th>Oxalate Fe (mg kg⁻¹)</th>
<th>Oxalate Si (mg kg⁻¹)</th>
<th>Electrical cond. (dS m⁻¹)</th>
<th>Carbonate (%)</th>
<th>Exch. Ca : Mg ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heath podzol (no columns)</td>
<td>A</td>
<td>1.84</td>
<td>2.0</td>
<td>1.48</td>
<td>1.63</td>
<td>80</td>
<td>74</td>
<td>90</td>
<td>0.03</td>
<td>0.16</td>
</tr>
<tr>
<td>Ae</td>
<td>1.81</td>
<td>1.9</td>
<td>1.49</td>
<td>0.93</td>
<td>5.8</td>
<td>33</td>
<td>30</td>
<td>110</td>
<td>0.01</td>
<td>0.16</td>
</tr>
<tr>
<td>Bs</td>
<td>1.90</td>
<td>1.9</td>
<td>1.50</td>
<td>0.87</td>
<td>4.5</td>
<td>141</td>
<td>142</td>
<td>181</td>
<td>0.02</td>
<td>0.18</td>
</tr>
<tr>
<td>E. incrassata (study tree) (partly completed pavement)</td>
<td>A</td>
<td>1.60</td>
<td>2.6</td>
<td>1.50</td>
<td>1.23</td>
<td>6.5</td>
<td>39</td>
<td>46</td>
<td>112</td>
<td>0.03</td>
</tr>
<tr>
<td>Bs</td>
<td>2.10</td>
<td>2.0</td>
<td>1.50</td>
<td>2.37</td>
<td>5.5</td>
<td>308</td>
<td>315</td>
<td>571</td>
<td>0.01</td>
<td>0.16</td>
</tr>
<tr>
<td>E. incrassata (fully formed single pavement)</td>
<td>Bt</td>
<td>28.86</td>
<td>147</td>
<td>1.71</td>
<td>26.60</td>
<td>20.5</td>
<td>1077</td>
<td>1045</td>
<td>1791</td>
<td>0.20</td>
</tr>
<tr>
<td>E. pleurocarpa (double pavement profile)</td>
<td>Bkt</td>
<td>14.60</td>
<td>1.55</td>
<td>18.30</td>
<td>13.7</td>
<td>498</td>
<td>445</td>
<td>1028</td>
<td>0.57</td>
<td>4.98</td>
</tr>
<tr>
<td>E. occidentalis (‘old’ lake side profile)</td>
<td>A</td>
<td>2.70</td>
<td>2.5</td>
<td>1.42</td>
<td>3.30</td>
<td>8.5</td>
<td>540</td>
<td>204</td>
<td>911</td>
<td>0.02</td>
</tr>
<tr>
<td>Bs</td>
<td>25.40</td>
<td>169</td>
<td>1.62</td>
<td>27.30</td>
<td>23.9</td>
<td>449</td>
<td>213</td>
<td>709</td>
<td>0.35</td>
<td>0.20</td>
</tr>
<tr>
<td>Bkt</td>
<td>25.69</td>
<td>119</td>
<td>1.55</td>
<td>28.40</td>
<td>22.8</td>
<td>708</td>
<td>315</td>
<td>694</td>
<td>0.76</td>
<td>1.32</td>
</tr>
</tbody>
</table>

All values are on a dry-soil mass basis. See also Fig. 4 for plots of changes in Al, Fe, Si and Th down the profiles.

* Horizon designations: A, upper sandy horizon above columns where present; Ae, bleached sandy horizon; Bs, weak sesquioxidic sandy horizon; Bt, clayey column formations in what was formerly an Ae horizon; Bk, carbonate-enriched Bs; Bkt, carbonate and clay enriched Bs.

† CU, Coefficient of uniformity as defined in the text.

#### (B) Clay and mineral contents of eucalypt profiles above those of podzol baselines

<table>
<thead>
<tr>
<th>E. incrassata (study tree) incompletely formed pavement</th>
<th>Carbonates (kg)</th>
<th>Total Al (kg)</th>
<th>Total Fe (kg)</th>
<th>Ox. Al (kg)</th>
<th>Ox. Fe (kg)</th>
<th>Ox. Si (kg)</th>
<th>Clay (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. incrassata fully formed single pavement</td>
<td>0.02</td>
<td>1.9</td>
<td>0.8</td>
<td>0.17</td>
<td>0.24</td>
<td>0.47</td>
<td>18</td>
</tr>
<tr>
<td>E. incrassata/pleurocarpa double pavement</td>
<td>32.28</td>
<td>113</td>
<td>3.6</td>
<td>0.12</td>
<td>0.18</td>
<td>0.04</td>
<td>129</td>
</tr>
<tr>
<td>E. occidentalis ‘old’ lakeside profile</td>
<td>49.46</td>
<td>61.4</td>
<td>29.6</td>
<td>1.22</td>
<td>1.16</td>
<td>2.92</td>
<td>488</td>
</tr>
<tr>
<td>54.71</td>
<td>115.3</td>
<td>73.0</td>
<td>2.93</td>
<td>1.16</td>
<td>2.53</td>
<td>1119</td>
<td></td>
</tr>
</tbody>
</table>

Values relate to quantities in slabs of soil 1 m² at surface and 3 m deep after subtraction of respective quantities in comparable podzol under heath. The data were obtained from integration of values of seven to nine sampling stations down the respective profiles.

are more or less commensurate with percentages of precipitated clay (cf. entries in Table 1A) as would be expected from progressive infilling of pore spaces of the original sandy matrix as pavements are constructed. (e) Values for CUs (see Table 1) show marked increases in sorting coefficients (decreasing particle sorting) as one would associate with introduction of clay in an aeolian sand deposit. Plots of cumulative weight percentage axis essentially in proportion to the amount introduced clay. (f) Comparisons of increases in clay and mineral contents in eucalypt profiles relative to podzol baselines (Table 1B) provide evidence of absolute increases in clay and carbonates and their respective principal constituents (see relevant entries, Table 1B). However, there are lesser proportional increases in the amorphous (oxalate extractable) constituents of the clay, although these represent a small fraction relative to well-crystallized clays.

### Al, Fe and Si analysis of leafy shoot samples of eucalypt and non-eucalypt species

This comparison showed Al concentrations in eucalypt and non-eucalypt species of 26 ± 3.3 mg kg⁻¹ versus 63 ± 26, respectively, Fe concentrations of 36 ± 5.84 versus 58 ± 5.8, respectively, and Si concentrations of 56 ± 15 versus 63 ± 7.3, respectively (same units). Statistical analysis recorded significantly higher values for Al (P < 0.01) in non-eucalypts than eucalypts but no large difference with respect to Fe and Si.

### pH profiles in a cutting through the dune

Figure 6 depicts a collage of photographs (fish-eye view) of the cutting through cleared land adjacent to the reserve. Spatial relationships are indicated between the single- and double-clay pavements laid down under the original eucalypt woodland (Stations 1–5) and the pavement-free area previously occupied by myrtaceous:proteaceous heath (Stations 5–7). A Krig-based interpolation of pH distribution across the face is
shown as a colour-coded contour map covering the pH range 4.5 through to 9.5. Extra sampling stations (dotted black lines in Fig. 6) were required to improve resolution of the marked transition in soil pH at the junction between pavemented (pH 7–10) and non-pavemented (pH 4.5–6.0) zones of the cutting.

While A horizons of heath and woodland were uniformly acidic (pH 5–6.5), subsoil pH increased by one to two units from the furthest-extending parts of pavement back several metres into fully formed pavement. Two regions under fully formed pavement were particularly alkaline (pH > 9.0; see dark blue colouration in Fig. 6) and contained >2 % total Ca and >1.8 % total Mg, mostly as carbonate around root material (data not shown). Corresponding values for Ca and Mg for sand under heath were <0.2 % and <0.1 %, respectively.

**Chemical composition of xylem saps from mallees and proteaceous species**

From August through to early October 2008 the upper soil remained well wetted and the majority of superficially located roots of the three eucalypts and laterals of a selection of Proteaceae in the top 30 cm of the profile yielded sufficient sap for analysis. This upper soil horizon dried out rapidly through to mid-November leading to most roots failing to yield sap. However, an unusually large rainfall event in November rewetted the profile to over 1 m depth and sap was again collectable from most roots. Summer weather patterns then dried profiles down to at least 1.5 m and, with xylem elements of most lateral roots of Proteaceae and R1 roots of eucalypts then becoming embolized, xylem sap could no longer be collected. By contrast, the xylem of most R2 roots in the 30- to 80-cm zone remained fully hydrated and a large proportion of these roots remained amenable to sap collection.

Table 2A shows mean concentrations of key elements in wet season sap samples from Proteaceae and each of the three eucalypts. Differences in values for the eucalypts (upper part of Table 2A) proved to be relatively small, and when this set of 12 samples were compared with a corresponding set of 12 from Proteaceae (see items 2 and 3 in Table 2A) only small differences in composition emerged. Levels of mineral elements in all samples were generally moderate-to-low, as would be expected if the roots concerned were transporting...
from well-wetted upper soil profiles that are noticeably deficient in most nutrients (see data of Table 1).

A comparison of wet season (R1 and R2 roots) with dry season (R2 roots) samples from *E. incrassata* (Table 2B) provided evidence of substantial differences in respect of certain elements. Thus, sap samples from R2 roots during summer averaged at 100 times more concentrated in Al ($P = 0.008$) and 5–15 times more concentrated in Si, Mg and Fe ($P = 0.008, 0.024$ and $0.310$, respectively) than in the case of roots (R1 and R2) harvested in winter. By contrast, concentrations of Ca, K and Na were closely similar between winter and summer samples. A noticeable feature of the summer data was the large-scale variability in values for Al and Mg across the R2 root samples assayed (see large standard errors for these elements in Table 2B). There were insufficient samples taken over the season to determine whether this variability related to night versus day sampling.

Organic acid analyses on nine samples of sap showed citrate to be the main component ($1520 \pm 510 \mu M$) followed by malate ($139 \pm 46 \mu M$) and oxalate ($30 \pm 15 \mu M$). Other components at much lower concentrations were fumarate, malonate, *trans*-aconitate and shikimate. Summer (R2 root) samples tended to be particularly high in citrate.

Chemical composition of bore water sampled within the Reserve (Table 2A) showed low levels of Al and Fe compared with Si and much high levels of Ca, Mg and particularly Na. Interestingly, Mg is noticeably higher than Ca in both bore water and dry season xylem sap. Workers such as Rengasamy and Olsson (1991) and Chartres (1993) have linked the marked accumulation of Mg in column material relative to Ca (see also the data in Table 1) to rising saline ground waters, although these workers failed to specify the mechanisms involved or indeed to implicate mining activities of roots in the process.

We regard it invalid to compare deep bore water composition with xylem sap since the bores were located some distance from where our trees were sampled, although it was likely that the taproots concerned would have been sourcing lenses of fresher water perched on top of saline ground waters of similar constitution.

**DISCUSSION**

The rooting architecture of the mallee *E. incrassata* is dimorphic [see detailed accounts by Pate *et al.* (1995) for woody Proteaceae] and basically as exhibited by the majority
of the over one thousand woody native Australian species so far examined (see Pate and Dixon, 1996; Pate and Bell, 1999). Root systems of this type consist of a platform of super-
ficially extending lateral roots and one or more deeply pen-
etrating tap roots. Both of these classes of root attach proximally to a junction region which in turn bears the aerial shoot system. In mallee eucalypts and many other fire-
resistant species, the shoot : root junction is markedly swollen and generally referred to as a lignotuber. It houses numerous latent epicormic buds from which new shoots become established after death or damage of an earlier estab-
lished shoot system (e.g. see study on coppicing of the oil mallee Eucalyptus kochii by Wildy and Pate, 2002).

The lateral root system of E. incrassata consists of two dis-
tinct tiers of roots. Upper-tier roots (R1) remain fully hydrated and presumably active in uptake of water and nutrients during the wet season, but then mostly become embolized and cease to transport water. By contrast, lower-tier roots (R2) typically remain well hydrated through the summer when they suppo-
sedly function in delivering tap-root-derived water and mineral elements to developing columns and soil profiles below pavements.

The extensive literature on patterns of hydraulic redistribu-
tion in roots of phreatophytic woody species portrays a general capacity for reversible lateral and vertical flows between different regions of the roots systems of a variety of species. Flows are depicted as being driven primarily by gra-
dients in water potential, as dictated by availability of vadose versus ground water, transpiration activity and sharing of water resources with shallow-rooted understorey species. A variety of techniques has been used to study such phenomena, particularly heat pulse studies (see Caldwell and Richards, 1989; Horton and Hart, 1998; Burgess et al., 2000; Burgess and Bleby, 2006), deuterium-based stable isotope assessments of changing water sources and sinks in a system (Dawson, 1993; Dawson and Pate, 1996; Pate and Dawson, 1999; Ludwig et al., 2003) and water-balance studies of soil and roots and transpiring shoots as evaluated for the oil mallee Eucalyptus kochii by Wildy et al. (2004). Currently there is no information on seasonal and daily depend-
dence of the study species on ground water as opposed to recent rain and water stored in different regions of the soil profile, nor has the extent been evaluated to which shoot tran-
spiration competes with upper- and lower-tier roots for ground water accessed by tap roots. However, preliminary studies on xylem architecture of the species (J. S. Pate, unpubl. res.) show greater xylem vessel diameters in lower than upper-tier 

lateral roots and a marked contrast between the broad and long vessels typical of sinker roots and the shorter vessels of lesser diameter in the trunk and upper parts of the lignotuber. Effects such as these may well set the pattern for hydraulic flows between sources and sinks, as proposed by Pate et al. (1995) for shrub and tree Proteaceae. The recent proposal by Burgess and Bleby (2006) that redistribution of soil water amongst lateral roots of tree eucalypts (Eucalyptus wando-

oa and E. salmonophloea) is driven by water resources and reversible xylem pathways in trunk tissues, may well find par-

allels in the respective water relationships of lignotuber, shoot and the two classes of root in E. incrassata. In any event, gross sectionings of lignotuber reveal an intricate twisting and

anastomosing of vascular tissue, including a potential for new shoots arising on a lignotuber to become connected with the vasculature of the parent plant. Exchange processes between tuber and surrounding organ are therefore likely to be exceedingly complex, possibly with certain pathways util-

ized much less effectively than others because of high hydra-

ulic resistances within the system. Within this scenario one might find an explanation of why R2 roots but not R1 roots remain hydrated and presumably actively transporting during summer.

The estimates of rates of clay deposition under E. incrassata 
on the crest of the dune used data for a single tree which cur-
rently carries approx. 3 tonnes dry weight of self-generated pavement material, or the equivalent of 700 kg of clay. Relating this amount of pavement to current live biomass would be misleading since most columns of the pavement were associated with long-decayed remains of roots and there-
fore constructed much earlier in the life of the tree. Accordingly it was necessary to recourse to information for the nine live lower-tier roots which proved to be currently active in constructing columns across the root catchment. With a mean weight of 9 kg of column material (2-1 kg of 

clay) associated with each root and growth-ring estimates suggesting that the roots concerned are 8 years old, their collective depositional performance would amount to 19-5 kg of clay or the equivalent of 2-4 kg of clay per tree per year. This would equate to an annual rate of production of 62 g clay m$^{-2}$, so the tree would be expected to have taken 300 years to accumulate the 700 kg of clay material now present across its 40 m$^{2}$ root catchment.

In the mid-slope of the dune where columns under mallee woodland were consolidated into virtually continuous pavement, 130 kg m$^{-2}$ of neoformed clay had accumulated. Assuming annual rates of clay deposition to be similar to those of the study tree, the eucalypts involved at such locations would have completed their collective programmes of pavement construction in just over 2000 years. This time scale would appear reasonable for eucalypt colonization of a dune supposedly laid down 10 000 years ago.

Moving to the lowermost regions of the lake-facing side of the dune, colonization by the tree eucalypt E. occidentalis was found to be associated with massively paved pavement profiles carry-
ing 1120 kg m$^{-2}$ of clay. Events and time scales are unclear in this situation, although it would be reasonable to expect much faster rates of clay deposition by E. occidentalis in view of its large size and presumed capacity for monopolizing water and nutrients.

Preliminary investigations of the fine structural features of nascent columns, demonstrate intimate spatial relationships between clay particles, fine plant roots, fungal mycelia and other micro-organisms. While it is impossible to distinguish at this stage which biotic elements might be formative, as opposed to coincidentally associated with developing pav-
ements, the high density of mycelial filaments close to fine eucalypt rootlets would suggest these to be major metabolic sinks for the chelated and free solutes being released by roots. However, the possibility should not be ruled out that specialized bacteria are involved in clay biogenesis, similar to those described in the in vitro studies of Tazaki (2005, 2006). Further uncertainty sur-
rounds the chemical environment in which clay biogenesis
operates, although our pH measurements on nascent columns indicate a mildly acidic environment in which microbial decomposition of low molecular weight complexes might initially yield ferrihydrite- and imogolite-type compounds (see Farmer and Lumsden, 2001).

The present analyses of chemical and physical composition of soil cores has provided dramatic evidence of the extent to which profiles under eucalypts become modified in comparison to those under closely adjacent myrtaceous:proteaceous heathland. Grossly elevated total levels of Al, Fe and Mg and amorphous levels of Al, Fe and Si turn out to be overt hallmarks of clay biogenesis and, as pores in developing columns become progressively occluded, bulk densities increase proportionately. Furthermore, particle size distribution analyses (CU and P plots) conducted for a range of sites across the dune showed high levels of sorting for all profiles and horizons involving unmodified sand. This is exactly as expected for uniform depositional regimes during aeolian deposition of the dune. By contrast, particle distributions in clayey horizons were exactly as expected of clay having been introduced into an already sorted sand deposit.

The present data on chemical composition of profiles showed marked progressive enrichment in Ca and Mg carbonates beneath pavements with pH values accordingly elevated. This contrasts forcibly with the situation under heath where, despite mild podzolization, mineral statuses remain more or less as expected of the original quartzitic sand dune deposit.

An interesting parallel to the contrasting situation found in mallee woodland versus heath at Chillinup has been recorded by Berger et al. (2006) in their studies of deep mining of Ca and alkalization of soil profiles by European beech (Fagus sylvatica) versus the strongly acidifying effects of cohabiting spruce (Picea abies). Regardless of whether mallee or beech is the responsible agency, long-term deep-mining of and subsequent deposition of divalent cations as carbonates in upper soil profiles would inevitably result in acidification of basement profiles and associated ground waters. Indeed, such effects are already evident in the marked association between acidic ground water and certain eucalypts at landscape and regional scales in semi-arid ecosystems across southern Australia (D. Gray, CSIRO, Australia, pers. comm.). Included here also would be the presence of highly acidic lakes draining from large bodies of eucalypt vegetation in eastern and coastal regions of south-west Western Australia.

The primary aim of this research was to determine the mechanisms which drive such massive accumulations of elements such as Al, Fe, Mg, and Ca under stretches of mallee on the dune at Chillinup. Xylem sap analyses provide key evidence in this connection. Thus, during the wet season when water and mineral element flows are expected to be predominantly from mineral-depleted upper soil layers towards transpiring tree or shrub, composition of sap from lateral roots is not noticeably different from that of eucalypt or proteaceous species. Then, during the dry season, sap from column-forming eucalypt roots becomes grossly enriched with Al and Mg, as is expected of deep mining and uplift by tap roots at times when trees have become highly dependent on ground water. At such times upper-tier laterals had mostly become embolized and presumably only marginally active as sources and sinks for water.

Using the estimate of 10 kg per year for pavement formation by the present study tree and a value of 2 % Al for its pavement material, one would conclude that uplift of Al by the tree would be equal to 200 g per year. Then assuming that Al concentration in xylem sap of lower-tier laterals was at the estimated mean value of 30 mg L$^{-1}$, 6700 L of xylem sap would need to be uplifted to meet this target value for Al. Interestingly, this amount of water is the equivalent of approx. 30 % of the mean annual rainfall for the root catchment, suggesting that pavement formation might comprise a relatively important item in the water transport budget of trees.

An alternative approach is to consider relationships between current standing biomass of the tree (237 kg dry weight) and that of the pavement formed over its life (2900 kg dry weight). With a mean Al concentration in tree dry matter of 26 mg kg$^{-1}$ and one of 2000 mg kg$^{-1}$ in pavement, the resulting current pools of Al are 6.2 g in the tree versus 58 kg in the pavement. On this basis, one would require the equivalent of almost 10000 times the current aluminium pool of the tree to be turned over to meet the observed increment in pavement Al. This is clearly an unreasonably high rate of transfer, whether it be by internal cycling of Al via shoots or by shedding and decomposition of biomass. This is particularly the case for woody species of semi-arid environments whose net annual productivities would be expected to be of the order of only 10 % of current standing biomass (see data for banksia woodlands by Pate and Bell, 1999).

Applying the same scenario to our hypothesis of hydraulic uplift of Al sourced from the lacustrine deposit, our mean Al concentration of 30 mg L$^{-1}$ in xylem sap of R2 roots requires a targeted uplift of 1.93 million litres of water during the life of the tree. Over a presumed life of 300 years, this would equate to uplift of 18 L per day or 70 L per night in summer. These values lie well within the range of values of 50–235 L per night for hydraulic uplift recorded for other woody tree species by Emerman and Dawson (1996) and Ludwig et al. (2003).

The extensive literature on ability of plants to take up, transport and accumulate Al, Fe and Si has suggested that such abilities extend widely across a broad range of flowering plants including Myrtaceae (see Jansen et al., 2002; van Hees et al., 2004; Hinsinger et al., 2006; Kim and Guerinot, 2007; Ma et al., 2007). In certain cases, where hyperaccumulation of Al is recorded, the element has been shown to be transported in chelated form, with citrate as the prime mediator (e.g. see Jansen et al., 2002; Watanabe and Osaki, 2001). Citrate values for xylem sap in the present study concur with this and further suggest that the same ligand is involved in the chelation and transport of Fe and Th identified in column and xylem samples.

It is interesting to note that the three study eucalypts at Chillinup contained only slightly different levels of Al, Fe and Si in their leafy shoots than those in non-eucalypt flora at the site (J. S. Pate and W. H. Verboom, unpubl. res.). The relatively low levels of Al recorded further indicate that none of the species analysed are hyperaccumulators of the element (see extreme values recorded for species referred to in the literature cited in the previous paragraph). As a corollary to the above, delivery of such large amounts of Al to sites of clay formation must be very specifically targeted in space and time. Here one might find an interesting scenario for
exploring mechanisms for selective uptake and transport in the plant body of a woody species.

It should be noted that a large body of information already exists concerning neof ormation of clay in soil profiles (see review of Wilson, 1999). In this context, Chittleborough and Oades (1980) have rejected differential weathering of strictly localized materials to explain texture contrast formation, but still entertain the possibility of illuviation from upper horizons. However, other workers such as Brewer (1968) and Oertel (1974) have been unable to find evidence for the latter in the texture-contrast profiles they studied and concluded that the clay must have formed in situ. In the present situation at Chillinup, initial levels of clay and weatherable minerals in the relatively isotropic quartzitic sand of the dune are clearly insufficient to account for the massive deposition of clay under mallee woodland at the site. At the same time information on the fine structure of depositions, bulk density and particle sorting provide convincing evidence of targeted introduction of neoformed clay associated specifically with lateral root pavements of mallee. Within this scenario, we strongly favour a sourcing of raw materials by deep mining by roots.

Finally, this, and earlier papers (Verbom and Pate, 2006b; Pate and Verbom, 2009) describing pavement morphologies of eucalypts, raise the intriguing question of how columns are spatially organized in such regular conformation and depth in a profile. Judging from the present study tree, spaces between earlier formed columns are progressively filled by subsequent column-forming events and the same principles presumably apply over extended periods until a virtually continuous clay layer has formed with constituent columns contiguous to the point of coalescence. The end situation bears a resemblance to the columnar-like patterns of mats of geologically ancient algal stromatolites encountered in certain marine embayments. Searching for a commonality in mechanisms generating similar effects across the biological scene might well prove to be a fascinating exercise.

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LITERATURE CITED


