100 YEARS Journal of Ecology

British Ecological Society

Journal of Ecology doi: 10.1111/j.1365-2745.2012.01962.x

Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot

Etienne Laliberté[1]*, Benjamin L. Turner[1,2], Thomas Costes[1,3], Stuart J. Pearse[1], Karl-Heinz Wyrwoll[4], Graham Zemunik[1] and Hans Lambers[1]

[1]*School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley 6009, Australia;* [2]*Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama;* [3]*Agrocampus Ouest, Centre d'Angers, Institut National d'Horticulture et du Paysage, 2 rue André Le Nôtre, 49045 Angers Cedex 01, France; and* [4]*School of Earth and Environment, The University of Western Australia, 35 Stirling Highway, Crawley 6009, Australia*

Summary

1. The classical model of long-term ecosystem development suggests that primary productivity is limited by nitrogen (N) on young substrates and phosphorus (P) on older substrates. Measurements of foliar and soil nutrients along soil chronosequences support this model, but direct tests through nutrient-addition experiments are rare.

2. We conducted a nutrient-limitation bioassay using phytometer species grown in soils from five stages of a >2-million-year dune chronosequence in south-western Australia. This long-term chronosequence is located within a region of exceptionally high plant species diversity and has not been previously studied in the context of ecosystem development.

3. Growth of unfertilized phytometers, a proxy for primary productivity, peaked on young soils (hundreds to a few thousand years) and then declined steadily on older soils. This decline was linked to P limitation, and its rapid appearance (< 7000 years) compared to other sequences reflects the low P concentration in the parent material. As predicted, growth of canola was N-limited on the youngest soil (stage 1), co-limited by multiple nutrients in stage 2 and increasingly P-limited thereafter.

4. Growth of wheat was P-limited from stage 2 onwards, yet on the youngest soil it was co-limited by potassium (K) and micronutrients – most likely iron (Fe). Nitrogen addition also decreased the root:shoot ratio of wheat such that shoot growth was higher than in the control. We attribute these responses to a parent material that is very low in K and N and strongly alkaline (pH [H_2O] > 9), being of a marine origin (i.e. carbonate dunes). Fe is poorly soluble at high pH and K likely plays a role in the secretion of Fe-mobilizing exudates from wheat roots.

5. *Synthesis*. Our results provide strong support for the long-term ecosystem-development model, particularly with regard to the appearance of P limitation and associated declines in productivity. However, our study also shows that N cannot be assumed to invariably be the most important limiting nutrient in young soils, and it is unlikely to be the only limiting nutrient in calcareous soils. This south-western Australian long-term chronosequence provides an excellent opportunity to explore edaphic controls over plant species diversity.

Key-words: co-limitation, ecosystem decline, ecosystem progression, ecosystem retrogression, pedogenesis, plant–soil (below-ground) interactions, productivity, soil age, stoichiometry, succession

Introduction

Soil and ecosystem development begin following the deposition of a new substrate (e.g. coastal dune, lava flow, moraine). Over relatively short time-scales (decades to centuries), ecosystem properties such as net primary productivity increase and reach maximum values (Odum 1969). However, in the absence of major disturbances over much longer periods (thousands to hundreds of thousands of years), this progressive phase is followed by gradual declines in many ecosystem properties – a phenomenon termed 'ecosystem retrogression' (Peltzer *et al.* 2010).

*Correspondence author. E-mail: etienne.laliberte@uwa.edu.au

Ecosystem progression and retrogression are causally linked to changes in nutrient availability, particularly nitrogen (N) and phosphorus (P), during long-term soil development (Peltzer *et al.* 2010). The classical pedogenesis model (Walker & Syers 1976) proposes that N is initially limiting, because it is largely absent from parent material and comes from symbiotic N_2 fixation or atmospheric deposition (although recent research suggests that bedrock N input may be significant in some systems; Morford, Houlton & Dahlgren 2011). On the other hand, P is derived primarily from weathering of parent material and its total amount and availability decreases with time until it eventually becomes the limiting nutrient in old soils (Beadle 1954; Walker & Syers 1976; Vitousek & Farrington 1997). In intermediate-aged soils, where nutrient supply may have equilibrated relative to plant demand, N and P may be co-limiting (Walker & Syers 1976; Vitousek & Farrington 1997). This may occur if, for example, P supply limits rates of N_2 fixation, or if P limits productivity such that N is increasingly lost (Vitousek *et al.* 2010).

Well-studied long-term soil chronosequences around the world have provided evidence that ecosystem progression is followed by ecosystem retrogression (Wardle, Walker & Bardgett 2004). In many long-term chronosequences (e.g. Walker & Syers 1976; Thompson 1981; Crews *et al.* 1995; Chadwick *et al.* 1999; Selmants & Hart 2010), shifts in the total amounts or availability of soil N or P with pedogenic development are consistent with predictions from the Walker & Syers (1976) model. That said, because the availability of multiple nutrients change in concert during ecosystem development (Walker & Syers 1976; Chadwick *et al.* 1999), soil data alone cannot confirm the type of nutrient limitation (e.g. N. vs. P limitation). Likewise, increasing foliar N:P ratios with ecosystem development (Richardson *et al.* 2004) provide only circumstantial evidence for shifts from N to P limitation, since these ratios are not always reliable indicators of nutrient status (Vitousek, Turner & Kitayama 1995; Craine, Morrow & Stock 2008) and need to be calibrated against results from nutrient-addition experiments. This is especially true when foliar N:P ratios of different species are compared among chronosequence stages (e.g. Richardson *et al.* 2004).

Nutrient-addition experiments provide the only direct evidence of the type and degree of nutrient limitation. However, evaluating nutrient limitation at the community or ecosystem scale presents significant challenges because species occupying infertile habitats typically do not respond strongly to fertilization (Chapin, Vitousek & Cleve 1986). As a result, the potential productivity of infertile ecosystems following nutrient addition will be largely realized through shifts in species composition (Chapin, Vitousek & Cleve 1986), which can take many years to develop. This problem can be resolved by fertilizing the same phytometer species growing in soils from different ecosystem-development stages, either in the field or *ex situ*. Nutrient-addition field experiments have so far only been conducted along the Hawaii chronosequence (Vitousek *et al.* 1993; Herbert & Fownes 1995; Vitousek & Farrington 1997; Harrington, Fownes & Vitousek 2001). Therefore, despite the central role of nutrient limitation to causal explanations of ecosystem progression and retrogression (Peltzer *et al.* 2010), our ability to conclusively state this phenomenon is associated with shifts from N to P limitation remains limited.

We conducted a nutrient-limitation bioassay (Beadle 1954; Van Duren & Pegtel 2000; Köhler *et al.* 2001) using soils from a Quaternary dune chronosequence in Western Australia (encompassing much of the last *ca.* 2.5 million years) to provide a direct test of the Walker & Syers (1976) model. We grew three phytometer species [canola (*Brassica napus*), white lupin (*Lupinus albus*) and wheat (*Triticum aestivum*)] in soils from five chronosequence stages (ranging from very young soils to soils >2 million years old) treated as follows: + water only, + N, + P, + potassium (K), + other nutrients (but not N, P or K) or + all nutrients. The three phytometer species were selected to represent distinct nutrient-uptake strategies: canola is non-mycorrhizal (Smith & Read 2008), wheat is mycorrhizal (Smith & Read 2008), while white lupin makes cluster roots (Gardner, Parbery & Barber 1981), fixes N (Layzell *et al.* 1979) and is weakly mycorrhizal (Bedmar & Ocampo 1986).

Our study introduces a number of novel aspects from previous work. The dune chronosequence used here has not previously been studied in the context of ecosystem development. Interestingly, it is located in a region with extremely high plant diversity (Lamont, Downes & Fox 1977; Hopper & Gioia 2004). The wet-winter, dry-summer Mediterranean climate of the study area is relevant because the generality of the Walker & Syers (1976) model has been challenged for water-limited ecosystems (Meixner & Singer 1985; Lajtha & Schlesinger 1988; but see Selmants & Hart 2010). The alkaline parent material is also significant given that pH strongly determines the availability of many nutrients, including P and iron (Fe; Marschner 1995). Our consideration of K is important because any rock-derived nutrient (not just P) could theoretically limit productivity during ecosystem development, as recently shown by strong K limitation of tree seedling and sapling growth in a tropical forest soil (Santiago *et al.* 2012; Wright *et al.* 2011). Finally, our experiment considers a greater range of chronosequence stages (here, five) than previous nutrient-limitation studies (three; Vitousek & Farrington 1997).

We hypothesized that:

1. productivity (as estimated by the growth of control plants in the unfertilized treatment) would increase rapidly from very young to intermediate-aged soils, and then decline gradually on older soils (Peltzer *et al.* 2010);

2. N would initially limit productivity on young soils, multiple nutrients would be co-limiting in intermediate-aged soils and P would be limiting in older soils (Walker & Syers 1976; Vitousek & Farrington 1997).

Materials and methods

STUDY AREA: THE JURIEN BAY DUNE CHRONOSEQUENCE

A long-term dune chronosequence is found in south-western Australia. The Swan Coastal Plain, a *c.* 400 km plain north and south of Perth, comprises a series of three main pedogenic-lithostratigraphic

associations that are generally interpreted as representing defined morphostratigraphic marine/dune systems and whose ages increase inland from the coast (McArthur & Bettenay 1974; Fig. 1). Soils used in this bioassay were collected along the Jurien Bay dune chronosequence, *c.* 225 km north of Perth, where all three main dune systems are present (Griffin & Burbidge 1990). The sequence covers a very long period of ecosystem development, extending over much of the Quaternary (the base of the Quaternary is taken as 2.59 Ma; McArthur & Bettenay 1974; Kendrick, Wyrwoll & Szabo 1991; Hearty & O'Leary 2008; Fig. 1).

Unlike the Cooloola sand dune chronosequence in eastern Australia (Queensland), where deep dunes were deposited as quartz sands with low carbonate ($CaCO_3$) content (Thompson 1981), the dune systems of the Swan Coastal Plain in south-western Australia have a more complex origin. The younger succession (Quindalup and Spearwood) have a primary carbonate-rich origin (McArthur & Bettenay 1974; McArthur 2004; Hearty & O'Leary 2008). In these, following dune deposition, carbonate is progressively leached from the upper horizons, eventually leading to a thick (i.e. many metres) siliciclastic sand overlying a karst weathering profile (McArthur & Bettenay 1974; Bastian 1996). The older succession (Bassendean) is often characterized by deep siliciclastic sands that are not associated with underlying carbonate lithologies, and appears to have been primarily deposited as quartz-rich sand lacking a significant carbonate component (Kendrick, Wyrwoll & Szabo 1991). Still, reference soils from all dune systems have a similar particle size distribution, with 73% (range 52–94%) coarse sand (200–2000 μm), 23% (range 3–43%) fine sand (20–200 μm), 2% (range 1–3%) silt (2–20 μm) and 2% (range 1–3%) clay (< 2 μm) (McArthur 2004).

The climate of the study area is strongly Mediterranean, with hot, dry summers and cooler, wetter winters. Mean annual rainfall (1981–2010) is 535 mm, 80% of which falls in the winter (May–September; Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/). There is little, if any, change in rainfall among the different dune systems, which are all within a short distance (*c.* 10 km) from the coast (See Fig. S1 in Supporting Information). Mean annual maximum temperature is 25 °C; the warmest mean monthly maximum temperature is 31 °C (February), while the coldest is 19 °C (July; Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/). Mean annual potential evapotranspiration (1961–90) is around 1500 mm (Australian Bureau of Meteorology, http://www.bom.gov.au/jsp/ncc/climate_averages/evapotranspiration/index.jsp). Fire is the main disturbance and fire intervals are typically < 30 years (Department of Conservation and Land Management 1995).

Contrary to other well-studied long-term chronosequences that go through forested stages (Wardle, Walker & Bardgett 2004), forest

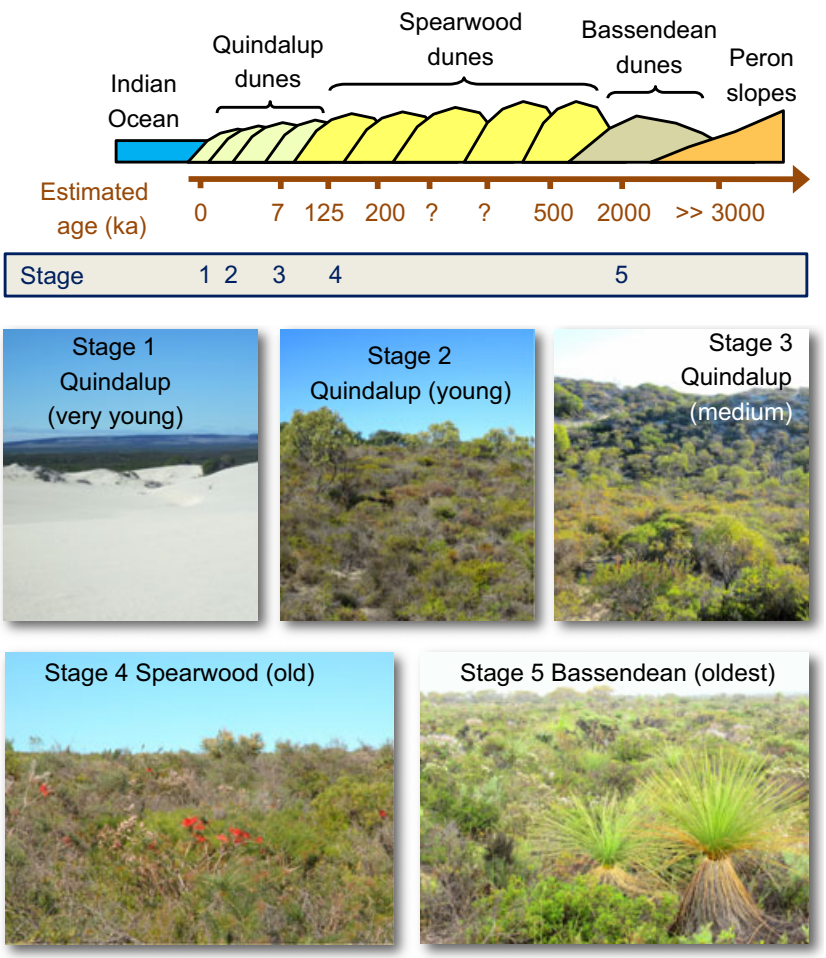

Fig. 1. West–east sequence of the three dune systems and subdivisions. Dune classification follows McArthur & Bettenay (1974) and Bastian (1996). Dune age generally increases from the coast (Indian Ocean) inland. The five chronosequence stages selected for the nutrient-limitation bioassay are shown. Around the study area, the Bassendean dunes meet the Peron Slopes, an ancient sandplain over laterite. The entire dune sequence can be found along a 10-km distance from the coast.

does not occur along the Jurien Bay dune sequence (Fig. 1). This is primarily due to the low and seasonal rainfall, although nutrient-poor sandy soils likely play a role. Plant species turnover is very high among the different dune systems, with Fabaceae (e.g. *Acacia* spp.) and Myrtaceae (e.g. *Melaleuca* spp.) common on younger dunes, and Proteaceae (e.g. *Banksia* spp.) on older dunes (Griffin & Hopkins 1990).

Below we describe the geology and soils of the three main dune systems that are recognized along the Jurien Bay chronosequence (Fig. 1).

Quindalup dunes

The Quindalup dunes are the youngest dunes of the sequence and are largely associated with the Holocene transgression (< 7 ka; McArthur 2004). Although a general west–east increase in age has been confirmed (Searle & Woods 1986), large unvegetated mobile dunes of very recent origin also occur at various distances from the coast (Fig. 1 and Fig. S1). McArthur (2004) sub-divided dune development into four distinct phases (Fig. 1) based on relief and soil development (organic matter penetration, i.e. depth of horizon A), with Q4 being the youngest (little to no organic staining) and Q1 the oldest (depth of A horizon = 50 cm). Due to their young age, carbonate dissolution is generally limited and the dune substrates typically lack karst attributes. Because of their relatively high carbonate content (*c*. 30–50% in horizons A–C; McArthur 2004), the sands are alkaline.

Spearwood dunes

The Spearwood dunes, inland from the younger Quindalup dunes, were formed in the middle to late Pleistocene (Kendrick, Wyrwoll & Szabo 1991). Bastian (1996) proposed that the Spearwood dunes can be divided into six dune sub-systems (Fig. 1) based on relief and associated heavy mineral assemblages. It is well known that the dune system increases in age to the east, with electron-spin resonance and amino-acid racemization techniques (Hewgill *et al*. 1983; Murray-Wallace & Kimber 1989) supporting Bastian's (1996) claims and demonstrating defined marine/dune associations extending into the middle Pleistocene. More recently, Hearty & O'Leary (2008) used amino-acid racemization techniques and reiterated that the Spearwood dunes increase in age eastward, and suggested ages of marine isotope stage (MIS) 5 e (*c*. 125 ka) up to MIS 13 (500 ka). In assessing the validity of these dates, the problems inherent in amino-acid techniques need to be borne in mind. Given the (paleo)climate setting and age of these sequences, foremost among these problems is the role of temperature changes in determining racemization reaction rates (Wehmiller & Miller 2000).

The Spearwood dunes consist of a calcarenite core (the Tamala Limestone) with calcretes and associated karst weathering profiles in the upper parts that are overlain by a variable thickness of siliciclastic sand (Tapsell, Newsome & Bastian 2003; McArthur 2004). The carbonate content of the Spearwood calcarenites ranges from 30% to 80% (60–70% on average; Bastian 1996), although some authors report higher values (85% on average; Hearty & O'Leary 2010).

Long-distance (> 800 km) wind transport from inland deserts has been proposed as an alternative hypothesis for the origin of the residual sand of the Spearwood dunes (Semeniuk & Glassford 1988; Hearty & O'Leary 2008, 2010). This hypothesis has been refuted on several grounds (Lowry 1976; Wyrwoll & King 1984; Bastian 1996, 2010; Newsome 2000; Tapsell, Newsome & Bastian 2003) and *in situ* weathering (with local remobilization) remains the most likely explanation. It is clear that the dissolution of a low-grade calcarenite (low carbonate content sand) leads to large amounts of residual sand, and *vice versa* (Bastian 1996). The depth of the residual sand generally increases eastward with dune age, possibly reflecting greater leaching, and can reach many metres (e.g. over 10–20 m) in depth (McArthur & Bettenay 1974; Tapsell, Newsome & Bastian 2003; Bastian 2010). However, surface erosion by rain or wind may strip the residual sand cover, especially from higher parts of dune ridges (Bastian 2010).

Bassendean dunes

The Bassendean dunes are the dune facies of the underlying Ascot Formation; the latter represents a series of depositional events associated with a prograding shoreline (Kendrick, Wyrwoll & Szabo 1991). The cover of white quartz-dominant sand can attain thicknesses of 15–20 m in some places (Kendrick, Wyrwoll & Szabo 1991), although our preliminary field observations suggest thinner accumulations of sand around Jurien Bay. Due to their considerable age (early Pleistocene to late Pliocene, i.e. > 2 Ma; Kendrick, Wyrwoll & Szabo 1991), the Bassendean dunes have lost their distinct dune topography over most of their width, and around Perth only the westernmost (youngest) dunes have a morphological expression (Bastian 1996).

Some authors have suggested that the Bassendean dunes may have originated as calcarenites (similarly to the Quindalup and Spearwood dunes), but that these have been leached for so long that carbonate has been entirely dissolved, leaving behind thick accumulations of quartz-dominant sand (McArthur & Bettenay 1974; Playford, Cockbain & Low 1976; Bettenay 1984; Bastian 1996). However, Kendrick, Wyrwoll & Szabo (1991) point to a clear distinction between the early Pleistocene (Ascot Formation), where sediments show higher quartz content, and the strong carbonate environment of the middle Pleistocene (Tamala Limestone), presumably reflecting higher carbonate productivity in the shelf environment from where the sand was sourced. Therefore, the Bassendean sands probably had much lower initial carbonate content than the Quindalup and Spearwood dunes.

As outlined above, the Bassendean dunes are considerably older than the Spearwood dunes (Kendrick, Wyrwoll & Szabo 1991; Bastian 1996; Tapsell, Newsome & Bastian 2003), although the details of the age structure of the three marine/dune successions remain to be firmly established. Over most of the Swan Coastal Plain, the Bassendean dunes have a width of *c*. 6–8 km (McArthur & Bettenay 1974), but near Jurien Bay they abruptly narrow to about 2 km at their widest (Griffin & Burbidge 1990).

SITE SELECTION

We selected four replicate sites in each of five distinct dune systems of increasing age [stage 1: mobile dunes (very young soils); stage 2: young Quindalup dunes; stage 3: older Quindalup dunes; stage 4: Spearwood dunes; stage 5: Bassendean dunes; Fig. 1], for a total of 20 sites (Fig. S1). Although accurate soil age data for the different stages are not available, we used previous soil classifications (Griffin & Burbidge 1990) in conjunction with our own field observations (i.e. soil characteristics, landscape features) to guide individual site selection.

Quindalup dunes (stages 1–3) can be easily distinguished from Spearwood dunes (stage 4), because the latter occur east of a distinct beach ridge that marks the transition between the Holocene and Pleistocene dunes (Fig. S1). Moreover, the Spearwood sands have a distinct yellow colour, which comes from the release of iron due to weathering of, for instance, ilmenite, garnet or amphibole (Bastian 2010). Similarly, the Bassendean dunes (stage 5) can be distinguished

from the Spearwood dunes (stage 4) by their landscape position (i.e. inland from the Spearwood dunes), lower relief and the colour of their sands, which are grey and never yellow (McArthur 2004).

Within the Quindalup dunes, mobile dunes (stage 1) were easily identified in that they are not yet vegetated (Fig. 1 and Fig. S1) and show no obvious soil development. Young Quindalup dunes (stage 2) were located near mobile dunes (stage 1), but were heavily vegetated and their soils were clearly more developed than those of stage 1. Older Quindalup dunes (stage 3) were selected on the basis of landscape position, i.e. just west of the Holocene–Pleistocene transition (Fig. S1). Although we are confident that the different stages can be ranked on the basis of relative age (Fig. 1), the current lack of high resolution numerical age control implies that a relatively large range of soil ages may exist with a given stage.

SOIL COLLECTION AND POTTING

Soil samples were collected in June 2011. In each of the 20 sites (Fig. S1), we laid down three 10-m transects (each spaced by 2–3 m) and collected ten 1.2-L soil samples per transect (one every metre), for a total of 30 soil samples per site. For each sample, the 0–30 cm layer was collected. Soil samples were bulked per site, oven-dried for 72 h, sieved (< 2 mm) and mixed thoroughly. Soil from each site was then potted into 18 ($= 3$ phytometer species $\times$ 6 treatments) 1.2-L sealed pots lined with plastic bags, each filled with 1.3 kg of dry soil per pot.

SOIL ANALYSES

Soil pH was determined in a 1:2 soil to solution ratio in both water and 10 mM $CaCl_2$ using a glass electrode. Total C and N were determined by automated combustion and thermal conductivity detection on a Flash EA112 analyser (CE Elantech, Lakewood, NJ, USA). Total P was determined by ignition (550 °C, 1 h) and acid extraction (1 M H_2SO_4, 16 h), with detection by automated molybdate colourimetry on a Lachat Quickchem 8500 (Hach Ltd, Loveland, CO, USA). Readily exchangeable phosphate (resin P) was determined by extraction with anion-exchange membranes (Turner & Romero 2009), while exchangeable cations were determined by extraction in 0.1 M $BaCl_2$ with detection by inductively coupled plasma optical-emission spectrometry (Optima 7300DV; Perkin Elmer, Shelton, CT, USA; Hendershot, Lalande & Duquette 2008). Carbonate was estimated by mass loss following HCl treatment (Loeppert & Suarez 1996).

PHYTOMETER SPECIES

We used three phytometer species with contrasting nutrient-acquisition strategies: canola (*Brassica napus* L. var. Tribune), white lupin (*Lupinus albus* L. var. Kiev mutant) and wheat (*Triticum aestivum* L. var. Wyalkatchem). Seeds of all species were sown on 22 June 2011. Prior to sowing, all soils were watered with deionized water up to 90% field capacity. Four, two and four seeds per pot were used for canola, white lupin and wheat, respectively, and subsequently thinned to three, one and three seedlings per pot.

NUTRIENT-ADDITION TREATMENTS

For each site/species combination, we used six treatments (Table S1): (i) deionized water only, (ii) +N, (iii) +P, (iv) +K, (v) other macro- and micronutrients, but not N, P and K (+Others) or (vi) complete nutrient solution (+All). Nutrients used for the different treatments are shown in Table S1. Calcium (Ca)-based nutrients were used for the +N and +P treatments, because we judged that Ca limitation was highly unlikely given the nature of the parent material (carbonate dunes) for most stages and the relatively high concentrations of Ca found in all soils, compared to that of other cations (McArthur 2004; see also Fig. 2f). Similarly, potassium chloride (KCl) was used as a K source in the +K treatment because Cl only becomes limiting in high-rainfall areas far from the sea (Xu *et al.* 1999), and all of our sites were located within 15 km from the Indian Ocean (Fig. S1). Consequently, Ca and Cl were omitted from the +Others treatment, such that combining the +N, +P, +K and +Others treatments would equate the +All treatment (Tables S1 and S2). We considered Cl toxicity to be unlikely since our addition of 175 mg Cl kg^{-1} soil in the +K and +All (Table S2) treatment was well below the critical toxic concentration of 600 mg kg^{-1} for wheat (Xu *et al.* 1999). Although initial soil Cl concentrations were not measured, we assumed that these were very low given the sandy nature of the soils.

Nutrient treatments started 1 week after sowing. Plants were fertilized and watered to 75% field capacity weekly for 6 consecutive weeks. Table S2 shows the total amount of nutrients supplied after 6 weeks of fertilization. Total amounts of nutrients are similar to those from a previous experiment involving white lupin and wheat (Pearse *et al.* 2006).

We estimated a 'leaf greenness index' on all lupin plants 5 weeks after the sowing date because white lupin showed signs of severe chlorosis in the high-pH soils of chronosequence stages 1–3. This greenness index was estimated by a Konica Minolta SPAD502+ chlorophyll meter on three fully developed leaves per lupin plant. This index is only used for comparative purposes, since meter readings were not calibrated against actual chlorophyll measurements.

Plants were harvested 8 weeks after sowing. Roots were washed over a 2-mm sieve and plants were partitioned into roots and shoots. Roots and shoots were oven-dried and then weighed separately.

STATISTICAL ANALYSES

We used log response ratios (LRR; Hedges, Gurevitch & Curtis 1999) as effect sizes for statistical analyses

$$LRR = \log_{10}\left(\frac{X_{\text{treatment}}}{X_{\text{control}}}\right)$$

where $X_{\text{treatment}}$ is the total (shoot + root) growth or root:shoot ratio of a plant receiving a particular nutrient-addition treatment (i.e. +N, +P, +K, +Others or +All) and X_{control} is the total growth or root:shoot ratio of the control plant (i.e. deionized water only). Log response ratios are commonly used in nutrient-addition experiments (Elser *et al.* 2007; Harpole *et al.* 2011). They tend to be normally distributed (Hedges, Gurevitch & Curtis 1999) and are easy to interpret: with a log base of 10 (as used here), a one-unit increase in LRR means that the growth (or root:shoot ratio) of a fertilized plant is ten times greater than the control; a LRR of 0 means no treatment response relative to the control.

Our design was a randomized block design (with blocks = individual sites). Therefore we used linear mixed-effects models (Pinheiro & Bates 2000) to test for effects of species, nutrient treatments, chronosequence stages and their interactions on LRRs, with random intercepts for sites. Models were fitted without intercept terms and 'treatment' contrasts were used to compare terms with the control (Pinheiro & Bates 2000). Whenever the variance clearly increased with the mean, LRR data were log-transformed or square-root transformed prior to analyses. Residuals were inspected visually to check model assumptions. When required, appropriate variance structures were specified in a second model, and both models were

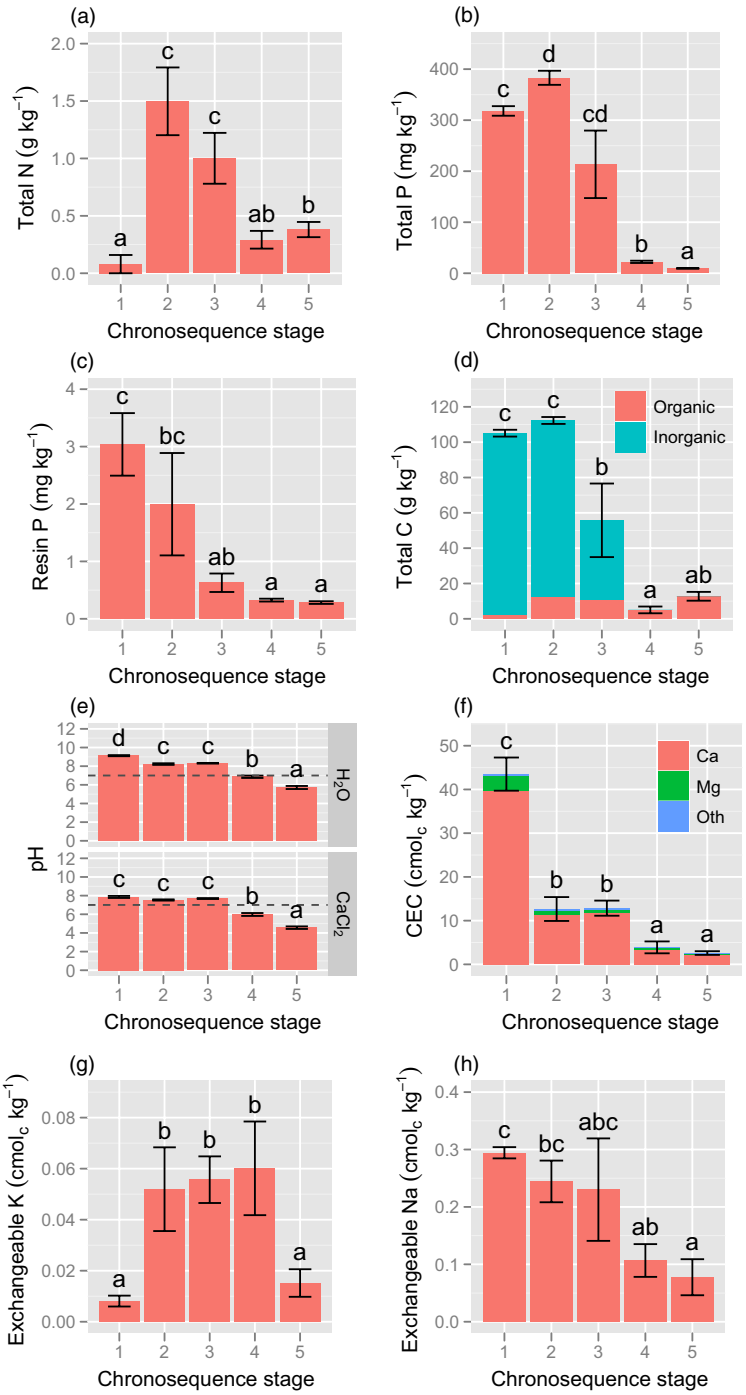

Fig. 2. Soil chemical properties across the five chronosequence stages. Means ± standard errors are shown. Different letters indicate significant ($P \leq 0.05$) differences among chronosequence stages based on 95% confidence intervals (CI). Dashed grey lines in panel (e) show pH = 7. CEC, cation exchange capacity.

compared using the Akaike Information Criterion (AIC) and likelihood-ratio tests (Zuur *et al.* 2009). When a main term was significant, *post hoc* Tukey tests were performed (Hothorn, Bretz & Westfall 2008). For analyses of soil data, no subsamples were taken and thus no site blocking factor was required; therefore, we used generalized least squares models (Zuur *et al.* 2009) and 95% confidence intervals (CI) of the LRRs for *post hoc* multiple comparisons. All analyses were conducted in the R environment (R Development Core Team 2011) using the nlme (Pinheiro, Bates & DebRoy 2010) and multcomp (Hothorn, Bretz & Westfall 2008) packages.

Results

SOIL CHEMICAL PROPERTIES

Very young soils (mobile dunes; stage 1) were almost devoid of total N, with values for three of four samples below detection level. As a result, total N in stage 1 was significantly ($P \leq 0.05$) lower than that in all other stages, except stage 4 (Fig. 2a). Total N peaked in stage 2 and then decreased in later stages

(Fig. 2a). Total N was much lower in stages 4 and 5 than in stages 2 and 3 (Fig. 2a).

Total P was highest in stages 1–3, and then strongly decreased in stages 4 and 5 (Fig. 2b). Stage 2 had higher total P than stage 1, while stage 4 had significantly higher total P than stage 5 (Fig. 2b). Total P in stages 4 and 5 was extremely low, particularly in stage 5 (9.5 mg kg^{-1} soil).

Resin P (i.e. 'readily available' P) reflected total P, with some differences (Fig. 2b, c). A notable exception was stage 3, in which resin P was very low (0.62 mg kg^{-1} soil) despite moderately high total P (213 mg kg^{-1} soil). Resin P in stage 3 was significantly lower than that of stage 1, while resin P values in stages 4 and 5 were significantly lower than those of stages 1–2.

Total C was very high in stages 1–2, lower but highly variable in stage 3 and lowest in stages 4–5 (Fig. 2d). However, total C in stage 3 was not significantly different than that of stage 5. Most of the total C in stages 1–3 was inorganic C from carbonate (Fig. 2d). Organic C, on the other hand, was invariably low and not significantly different among chronosequence stages ($P = 0.07$).

Very young soils (stage 1) were strongly alkaline (pH [H_2O] = 9.13; Fig. 2e) because of their high carbonate content (Fig. 2d). Soil pH decreased gradually with soil age and became acidic in stage 5 (pH [H_2O] = 5.8; Fig. 2e). As expected, soil pH measured in a $CaCl_2$ solution was consistently lower than pH measured in water by around one unit (Fig. 2e).

Cation exchange capacity (CEC) was high in stage 1, moderate in stages 2 and 3 and lowest in stages 4 and 5 (Fig. 2f). In all five chronosequence stages, Ca dominated CEC, while Mg was of secondary importance (Fig. 2f). Other exchangeable cations (i.e. Al, Fe, K, Mn, Na) were present in very low amounts (Fig. 2f). In particular, exchangeable K was extremely low across all stages, but was lower in stages 1 and 5 than in stages 2–4 (Fig. 2g). Exchangeable Na was the third most important cation; it was present in low amounts throughout, but was lower in older stages (4 and 5) than in very young soils (stage 1; Fig. 2h). Exchangeable Al, Fe and Mn were generally below detection levels.

GROWTH OF CONTROL PLANTS

Total biomass of control canola and wheat plants strongly increased from very young soils (stage 1) to stage 2 and then showed a gradual decline (Fig. 3). White lupin was not considered since it did not tolerate the high soil pH of the young Holocene dunes (stages 1–3), making it unsuitable for comparisons of soil fertility.

Wheat showed significantly greater total biomass than canola ($P \leq 0.0001$), and this effect was consistent among the different chronosequence stages (stage × species interaction; $P = 0.231$). Total biomass in stage 2 was significantly greater than that in all other stages (Fig. 3). Total biomass in the very young soils (stage 1) was not significantly different from that in the oldest stage (stage 5), but significantly lower than that in stages 3 and 4. Finally, biomass in stage 3 was significantly greater than that in stage 5 (Fig. 3).

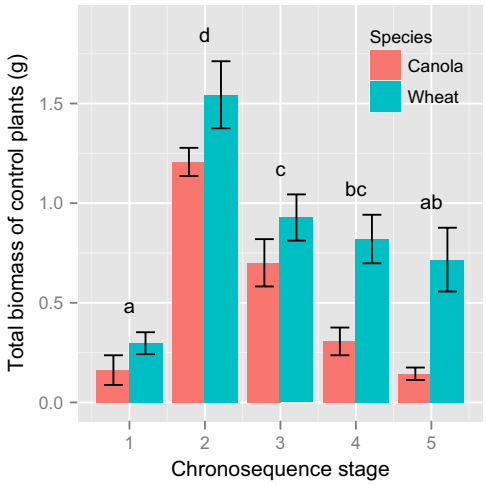

Fig. 3. Total biomass of control plants across the five chronosequence stages. Means ± standard errors are shown. Different letters indicate significant ($P \leq 0.05$) differences among chronosequence stages based on *post hoc* Tukey tests. Smoothed curves are generalized additive models (GAMs) for each species; grey bands around the curves are standard errors.

TYPE AND DEGREE OF NUTRIENT LIMITATION

Growth and root:shoot ratios of the three phytometer species responded differently to different nutrients in soils from different chronosequence stages (species × treatment × stage; $P \leq 0.0001$ for growth, $P \leq 0.05$ for root:shoot ratio). Therefore, separate analyses were conducted for each species/stage combination (Fig. 4; Figs S2 and S3).

Canola

Growth of canola was strongly N-limited in very young soils (stage 1), as shown by significantly ($P \leq 0.05$) higher total biomass with N addition (Fig. 4). Total biomass in the +N treatment was twice that observed in the control (Fig. 4).

In young and intermediate-aged soils (stages 2 and 3), growth of canola was limited by multiple nutrients. Indeed, no single nutrient-addition treatment increased growth relative to the control, and the +P treatment even had negative effects in soils from stage 2 ($P \leq 0.01$; Fig. 4). On the other hand, plants receiving all nutrients (+All treatment) had much greater total biomass than control plants ($P \leq 0.001$; Fig. 4), confirming the occurence of co-limitation.

In the two oldest stages (4 and 5), growth of canola was strongly limited by P. Total biomass of plants receiving additional P in soils from stage 4 and 5 was 1.9 and 4.3 times greater than that in the control ($P \leq 0.001$; Fig. 4), respectively.

Wheat

In very young soils (stage 1), growth of wheat appeared to be co-limited by K ($P \leq 0.05$) and nutrients other than K, N or P (+Others, $P \leq 0.001$; Fig. 4). Moreover, N addition also decreased the root:shoot ratio of wheat ($P \leq 0.001$; Fig. S2), such that shoot growth was higher than that of the control

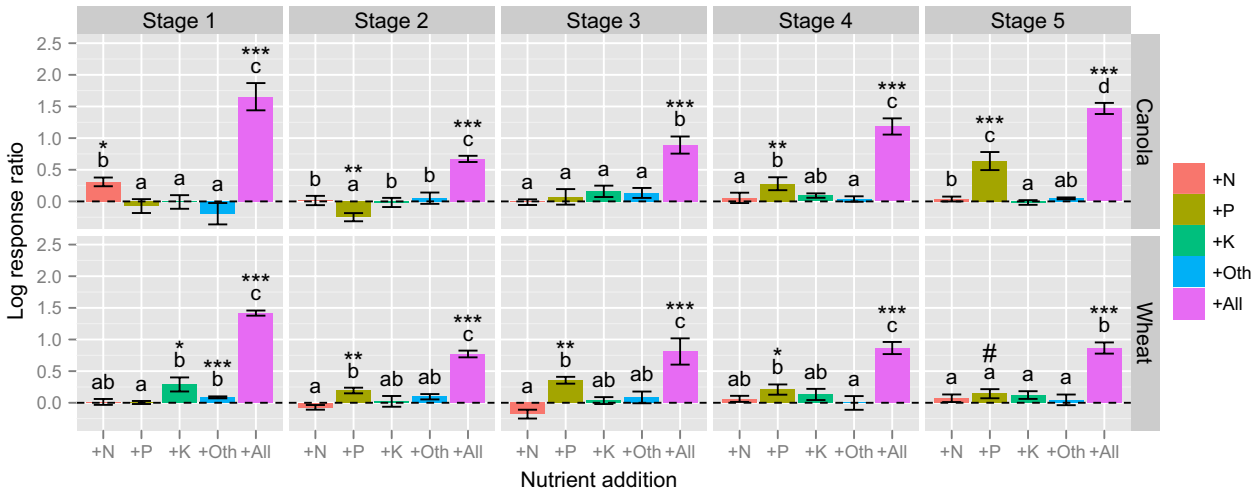

Fig. 4. Growth (shoot + root) responses (log response ratio) of canola and wheat to nutrient-addition treatments across all chronosequence stages. Means ± standard errors are shown. A one-unit increase in log response ratio represents a tenfold increase in biomass relative to the control. Stars indicate whether a treatment is significantly different from the control: $^{\#}0.1 \leq P < 0.05$; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. Different letters indicate significant differences ($P \leq 0.05$) between the treatments based on *post hoc* Tukey tests.

($P \leq 0.01$). In all other stages (stages 2–5), growth of wheat was P-limited, although this was marginally non-significant for stage 5 ($P = 0.076$; Fig. 4).

White lupin

White lupin did not tolerate the high-pH soils of stages 1–3, and showed signs of severe choloris in across all treatments in these younger soils. This is evidenced by the fact that even plants receiving the complete nutrient solution did not grow more than the control plants did (Fig. S3). Analyses of our 'leaf greenness index' showed a highly significant effect of chronosequence stage ($P \leq 0.001$), but no effect of nutrient treatment ($P = 0.644$) and no stage × nutrient treatment interaction ($P = 0.337$). The greenness index was much lower for stages 1–3 than for stages 4–5 ($P \leq 0001$; Fig. S4).

In soils from stage 4, growth of white lupin was P-limited ($P \leq 0.05$), yet surprisingly, plants receiving the complete nutrient solution did not grow more than control plants (Fig. S3). Even more surprisingly, P addition had negative effects' growth in soils from stage 5, whereas growth appeared to be limited by K ($P \leq 0.05$; Fig. S3).

In soils both from stages 4 and 5, white lupin plants receiving the complete nutrient solution showed much smaller growth responses, relative to the control, than canola or wheat. In those soils, total biomass of lupin plants in the +All treatment was only 1.1 and 1.5 times greater than that of the control plants, respectively (Fig. S3).

Discussion

ECOSYSTEM PROGRESSION AND RETROGRESSION

Our results provide strong support for the progression/retrogression model of ecosystem development (Peltzer *et al.* 2010), as well as associated changes in soil chemical properties and type/degree of nutrient limitation (Walker & Syers 1976). Assuming that the growth of phytometers without fertilizer addition (i.e. control plants) in soils from different chronosequence stages can be used as a proxy for potential primary productivity, our study shows that productivity peaks within a few hundred to a few thousand years and then declines steadily towards a 'terminal' state (stage 5; early Quaternary, > 2 Ma). As predicted, the onset of this decline was clearly associated with increasing P limitation (Walker & Syers 1976).

MECHANISMS UNDERLYING PHOSPHORUS LIMITATION

Vitousek *et al.* (2010) proposed six mechanisms that can drive P limitation in terrestrial ecosystems: (i) depletion due to prolonged weathering, (ii) formation of soil barriers that physically prevent roots from accessing deeper P pools, (iii) low P availability relative to that of other nutrients, (iv) low-P parent material, (v) P sinks, either biological (i.e. accumulation of biomass) or inorganic (i.e. sorption) and (vi) human-driven increases in other resources, particularly N. The strong reduction in total P with increasing soil age in our system points to a key role for weathering-driven P depletion, since total P in the oldest soils from our sequence was extremely low (9.5 mg kg^{-1}). The formation of root barriers obviously cannot explain our results because our bioassay approached used soil from the 0 to 30 cm layer. That said, a potential role of soil barriers during the early stages of ecosystem development in this system is likely, since weathering of carbonate dunes has led to the formation of secondary carbonate segregations, i.e. calcretes (McArthur & Bettenay 1974; Bastian 1996).

Low P availability relative to that of other nutrients may explain the apparent P limitation in intermediate-aged soils (i.e. stage 3), where resin P (representing 'readily available' P) was very low despite moderately high total P levels. Possible explanations for this result include conversion of more soluble Ca-P minerals to less soluble ones, or microbial immobiliza-

tion of available P. The low-P parent material certainly plays an important role in driving P limitation in this system. Indeed, very young soils contained less than half of the global average of 700 mg kg^{-1} for continental crust (Taylor & McClennan 1985). Overall, we suggest that the rapid appearance of ecosystem retrogression and associated P limitation in this system is best explained by the prolonged weathering of a low-P parent material, reinforced by conversion to less soluble P forms in intermediate-aged soils and occlusion of P in secondary minerals in the oldest, more acidic soils.

SOIL CHEMICAL PROPERTIES

Soil chemical properties measured in the surface (0–30 cm) horizon matched expectations from long-term ecosystem development well. As predicted (Walker & Syers 1976; Peltzer *et al.* 2010), total N strongly increased from stage 1 to stage 2 and then showed a gradual decline. Moreover, total P generally decreased with soil age, except for an increase of 64 mg kg^{-1} from very young (stage 1) to young soils (stage 2). This increase most likely reflects concentration of organic P in the surface (0–30 cm) from the activity of plants, given that organic C increased by 10 g kg^{-1} from stage 1 to stage 2. This would imply a soil organic C:organic P ratio of 154, which is well within the range found in soils in Australia and worldwide (Kirkby *et al.* 2011). The availability of P (as measured by resin P) also decreased with increasing soil age, most likely because of occlusion, sorption and conversion to less soluble P forms (Walker & Syers 1976). Cation exchange capacity (CEC) decreased with greater soil age, presumably reflecting leaching of Ca and downward movement of finer soil particles. The surface soil layer was gradually depleted in $CaCO_3$ during the first few thousand years of soil development, as it dissolves and precipitates lower down the soil profile (evidenced by the formation of secondary calcite; McArthur 2004). This was associated with a decrease in soil pH, due to the prolonged effects of rainfall and plant activity. Overall, these changes in soil properties are broadly consistent with those of other long-term chronosequences from around the world (Peltzer *et al.* 2010).

TYPE AND DEGREE OF NUTRIENT LIMITATION

As predicted by the Walker & Syers (1976) model, growth of canola was strongly N-limited in very young soils, co-limited by multiple nutrients in intermediate-age soils (stages 2, and stage 3 to a lesser extent) and strongly P-limited in old soils (stages 4 and 5). These results are consistent with those from previous field-fertilization studies of the tree *Metrosideros polymorpha* in the long-term Hawaii chronosequence (Vitousek & Farrington 1997) whose growth was N-limited in young soils (0.3 ka), co-limited by N and P in intermediate-aged soils (20 ka) and P-limited in old soils (4100 ka). The similarity in these results is remarkable given the vast differences in parent material and climate between the two study systems, and suggests that shifts from N to P limitation with soil age may be a general phenomenon.

Although our use of phytometers is a more direct way of assessing nutrient limitation than interpreting results from soil or foliar chemical analyses (Wheeler, Shaw & Cook 1992), this approach can still suffer from some limitations (Van Duren & Pegtel 2000). For example, nutrients differ greatly in their mobility (Marschner 1995), and low root occupancy of the soil volume by a phytometer plant could make it more responsive to the addition of a less mobile nutrient, such as P or Fe. Nevertheless, we believe that the clear shift from N to P limitation with soil age observed in canola, and the extremely low levels of soil N and P found in the youngest and oldest soils, respectively, provide strong support for the Walker & Syers (1976) model of soil development.

RESPONSES OF INDIVIDUAL PHYTOMETER SPECIES

Despite similar patterns of P limitation in old soils, wheat responded differently from canola to nutrient addition in very young soils (stage 1). Indeed, wheat responded strongly to K and micronutrients (and N to a lesser extent, as it reduced its root:shoot ratio and thus increased shoot growth), whereas canola only responded to N. The response of an individual plant species to multiple nutrients can occur via two distinct processes: 'multi-nutrient co-limitation', whereby multiple nutrients are present at very low levels, and 'biochemical co-limitation', whereby increases in one non-limiting nutrient improve the acquisition of a growth-limiting one (Arrigo 2005). As such, responses of wheat in very young soils (stage 1) to K, and N to a lesser extent, can be explained by the extremely low amounts of both nutrients, which were generally below detection levels. On the other hand, the response to other nutrients (+Oth) is likely to reflect the low availability of Fe at high pH (Marschner 1995). Biochemical co-limitation involving K and Fe may also be possible, since (i) K plays a key role in the release of Fe-mobilizing root exudates (i.e. phytosiderophores) by barley under Fe deficiency (Sakaguchi *et al.* 1999) and (ii) wheat, like all grasses, releases similar Fe-mobilizing exudates (Lambers, Chapin & Pons 2008). This shows that N cannot be assumed to invariably be the most important limiting nutrient in young soils and that it is unlikely to be the only limiting nutrient when the parent material is calcareous.

Different responses were also observed between canola and wheat in young soils (stage 2). Surprisingly, canola responded negatively to P addition in those soils, whereas wheat responded positively, despite high total soil P. The negative effect on canola suggests a signalling role of P beyond its role as nutrient (Raghothama 2000), whereby canola may adjust its growth based on internal P concentration, potentially leading to deficiencies in other nutrients. In addition, canola can readily access sparingly soluble Ca-P forms due to localized acidification behind the root tip coupled with malate exudation (Hoffland, Findenegg & Nelemans 1989; Hoffland 1992). However, wheat is far less effective than canola at acquiring P from sparingly soluble Ca-P forms (Pearse *et al.* 2007), which may explain why wheat but not canola was P-limited in those young, calcareous soils.

Contrary to wheat and canola, white lupin grew poorly in most soils, relative to control plants, even in the presence of a complete nutrient solution (+All). This was particularly obvious in the younger alkaline soils (stages 1–3), where all white lupin plants showed severe leaf chlorosis in all treatments. Previous studies identified white lupin as a calcifuge species (Tang *et al.* 1995; Liu & Tang 1999); however, Tang *et al.* (1995) found that chlorosis symptoms on new leaves disappeared after a few weeks, which did not occur in our study. The relatively poor growth of white lupin plants in acidic soils (stages 4 and 5), even those receiving the complete nutrient solution (+All), is difficult to explain, given our limited data. White lupin was selected as a phytometer species in this study due to its cluster roots (Gardner, Parbery & Barber 1981), a nutrient-uptake strategy shared by many native plants in our study system (Lambers *et al.* 2010). However, white lupin was obviously not well-suited to our bioassay approach and we therefore do not discuss results for this species any further.

Conclusions

Nutrient limitation is central to causal explanations of ecosystem progression and retrogression (Peltzer *et al.* 2010). Moreover, identifying the type of nutrient limitation is required to predict ecosystem responses to nutrient additions (e.g. atmospheric N deposition). However, direct evidence from nutrient-addition experiments for shifts from N to P limitation with long-term ecosystem development had been so far only available for the Hawaii long-term chronosequence (Vitousek *et al.* 1993; Herbert & Fownes 1995; Vitousek & Farrington 1997). In this study, we used another chronosequence with different parent material, climate and plant species composition and also found strong support for a general shift from N to P limitation during long-term ecosystem development, although our results also show that Fe and other micronutrients may be limiting in young calcareous substrates due to their poor availability (Marschner 1995).

Very few well-studied long-term chronosequences are known, restricting our ability to generalize causal links between nutrient limitation and long-term ecosystem development (Peltzer *et al.* 2010). The long-term dune sequence used here has never been previously studied in the context of ecosystem development and is therefore a valuable addition to the existing literature. Features that distinguish it from other sequences include a strong Mediterranean climate (Hopper & Gioia 2004) and exceptionally high levels of plant diversity at all spatial scales (Lamont, Downes & Fox 1977; Hopper & Gioia 2004). The type of nutrient limitation (e.g. N vs. P) influences competitive interactions between plants (Olde Venterink & Güsewell 2010) and may have implications for plant species coexistence through partitioning of different sources of N (McKane *et al.* 2002) or P (Turner 2008). How community-level properties such as species richness vary with long-term ecosystem development is an important gap in our knowledge (Peltzer *et al.* 2010; but see Wardle *et al.* 2008). As such, the long-term Jurien Bay chronosequence provides a strong opportunity to explore edaphic controls over plant species diversity.

Acknowledgements

We thank the Western Australia Department of Environment and Conservation (DEC), particularly Niall Sheehy, for their cooperation with soil collection and Tania Romero, Dianne de la Cruz and Luis Ramos for laboratory support. We also thank the Editor and two anonymous reviewers for comments that helped to strengthen the manuscript. Wheat seed was generously supplied by Daniel Mullan from Intergrain Pty Ltd, lupin seed by Bevan Buirchell from the Department of Agriculture and Food Western Australia (DAFWA) and canola seed by Matthew Nelson from Canola Breeders Western Australia Pty Ltd (CBWA). E.L. was supported by a Research Fellowship from the University of Western Australia and by an Australian Research Council (ARC) DECRA (DE120100352). H.L. acknowledges financial support from ARC.

References

Arrigo, K.R. (2005) Marine microorganisms and global nutrient cycles. *Nature*, **437**, 349–355.

Bastian, L.V. (1996) Residual soil mineralogy and dune subdivision, Swan Coastal Plain, Western Australia. *Australian Journal of Earth Sciences*, **43**, 31.

Bastian, L.V. (2010) Comment on Hearty, P.J. and O'Leary, M.J., 2008. Carbonate eolianites, quartz sands, and Quaternary sea-level cycles, Western Australia: a chronostratigraphic approach. *Quaternary Geochronology* **3**: 26–55. *Quaternary Geochronology*, **5**, 76–77.

Beadle, N.C.W. (1954) Soil phosphate and the delimitation of plant communities in eastern Australia. *Ecology*, **35**, 370–375.

Bedmar, E.J. & Ocampo, J.A. (1986) Susceptibilidad de distintas variedades de guisante, veza y lupino a la infeccion por Glomus mosseae. *Anales de Edalfologia Agrobiologia*, **45**, 231–238.

Bettenay, E. (1984) Origin and nature of the sandplains. *Kwongan: Plant Life of the Sandplain* (eds J.S. Pate & J.S. Beard), pp. 51–68. University of Western Australia Press, Nedlands, Western Australia.

Chadwick, O.A., Derry, L.A., Vitousek, P.M., Huebert, B.J. & Hedin, L.O. (1999) Changing sources of nutrients during four million years of ecosystem development. *Nature*, **397**, 491–497.

Chapin III, F.S., Vitousek, P.M. & Cleve, K.V. (1986) The nature of nutrient limitation in plant communities. *The American Naturalist*, **127**, 48–58.

Craine, J.M., Morrow, C. & Stock, W.D. (2008) Nutrient concentration ratios and co-limitation in South African grasslands. *New Phytologist*, **179**, 829–836.

Crews, T.E., Kitayama, K., Fownes, J.H., Riley, R.H., Herbert, D.A., Mueller-Dombois, D. & Vitousek, P.M. (1995) Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology*, **76**, 1407.

Department of Conservation and Land Management (1995) *Management Plan, Lesueur National Park and Coomallo Nature Reserve*. National Park and Nature Conservation Authority, Perth, Australia.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B. & Smith, J.E. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, **10**, 1135–1142.

Gardner, W.K., Parbery, D.G. & Barber, D.A. (1981) Proteoid root morphology and function in *Lupinus albus*. *Plant and Soil*, **60**, 143–147.

Griffin, E.A. & Burbidge, A.A. (1990) Description of the Region. *Nature Conservation, Landscape and Recreation Values of the Lesueur Area* (eds A.A. Burbidge, S.D. Hopper & S. van Leeuwen), pp. 15–24. Environmental Protection Authority, Perth, Western Australia.

Griffin, E.A. & Hopkins, A.J.M. (1990) Vegetation. *Nature Conservation, Landscape and Recreation Values of the Lesueur Area* (eds A.A. Burbidge, S.D. Hopper & S. van Leeuwen), pp. 25–38. Environmental Protection Authority, Perth, Western Australia.

Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E.S., Elser, J.J., Gruner, D.S., Hillebrand, H., Shurin, J.B. & Smith, J.E. (2011) Nutrient co-limitation of primary producer communities. *Ecology Letters*, **14**, 852–862.

Harrington, R.A., Fownes, J.H. & Vitousek, P.M. (2001) Production and resource use efficiencies in N- and P-limited tropical forests: a comparison of responses to long-term fertilization. *Ecosystems*, **4**, 646–657.

Hearty, P.J. & O'Leary, M.J. (2008) Carbonate eolianites, quartz sands, and Quaternary sea-level cycles, Western Australia: a chronostratigraphic approach. *Quaternary Geochronology*, **3**, 26–55.

Hearty, P.J. & O'Leary, M.J. (2010) Reply to Bastian, L.V.'s, Comment on Hearty, P.J. and O'Leary, M.J., 2008. Carbonate eolianites, quartz sands, and Quaternary sea-level cycles, Western Australia: a chronostratigraphic approach. *Quaternary Geochronology* **3**: 26–55. *Quaternary Geochronology*, **5**, 78–81.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, **80**, 1150–1156.

Hendershot, W.H., Lalande, H. & Duquette, M. (2008) Ion exchange and exchangeable cations. *Soil Sampling and Methods of Analysis* (eds M.R. Carter & E. Gregorich), pp. 173–178. Canadian Society of Soil Science, CRC Press, Boca Raton, Florida, USA.

Herbert, D.A. & Fownes, J.H. (1995) Phosphorus limitation of forest leaf area and net primary production on a highly weathered soil. *Biogeochemistry*, **29**, 223–235.

Hewgill, F., Kendrick, G., Webb, R. & Wyrwoll, K. (1983) Routine ESR dating of emergent Pleistocene marine units in Western Australia. *Search*, **14**, 215–217.

Hoffland, E. (1992) Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. *Plant and Soil*, **140**, 279–289.

Hoffland, E., Findenegg, G.R. & Nelemans, J.A. (1989) Solubilization of rock phosphate by rape. *Plant and Soil*, **113**, 155–160.

Hopper, S.D. & Gioia, P. (2004) The Southwest Australian Floristic Region: evolution and conservation of a global hot spot of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, **35**, 623–650.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Kendrick, G.W., Wyrwoll, K.-H. & Szabo, B.J. (1991) Pliocene–Pleistocene coastal events and history along the western margin of Australia. *Quaternary Science Reviews*, **10**, 419–439.

Kirkby, C.A., Kirkegaard, J.A., Richardson, A.E., Wade, L.J., Blanchard, C. & Batten, G. (2011) Stable soil organic matter: a comparison of C:N:P:S ratios in Australian and other world soils. *Geoderma*, **163**, 197–208.

Köhler, B., Ryser, P., Güsewell, S. & Gigon, A. (2001) Nutrient availability and limitation in traditionally mown and in abandoned limestone grasslands: a bioassay experiment. *Plant and Soil*, **230**, 323–332.

Lajtha, K. & Schlesinger, W.H. (1988) The biogeochemistry of phosphorus cycling and phosphorus availability along a desert soil chronosequence. *Ecology*, **69**, 24–39.

Lambers, H., Chapin, F.S. & Pons, T.L. (2008) *Plant Physiological Ecology*. Springer, New York, USA.

Lambers, H., Brundrett, M.C., Raven, J.A. & Hopper, S.D. (2010) Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil*, **334**, 11–31.

Lamont, B.B., Downes, S. & Fox, J.E.D. (1977) Importance-value curves and diversity indices applied to a species-rich heathland in Western Australia. *Nature*, **265**, 438–441.

Layzell, D.B., Rainbird, R.M., Atkins, C.A. & Pate, J.S. (1979) Economy of photosynthate use in nitrogen-fixing legume nodules: observations on two contrasting symbioses. *Plant Physiology*, **64**, 888–891.

Liu, A. & Tang, C. (1999) Comparative performance of *Lupinus albus* genotypes in response to soil alkalinity. *Australian Journal of Agricultural Research*, **50**, 1435–1442.

Loeppert, R.H. & Suarez, D.L. (1996) Carbonate and gypsum. *Methods of Soil Analysis, Part 3: Chemical Methods* (ed. D.L. Sparks), pp. 437–470. SSSA-ASA, Madison, WI, USA.

Lowry, D.C. (1976) Perth Basin yellow sand. *Search*, **7**, 393–394.

Marschner, H. (1995) *Mineral Nutrition of Higher Plants*. Academic Press, London, UK.

McArthur, W.M. (2004) *Reference Soils of South-Western Australia*, 2nd edn. Department of Agriculture (WA), Perth, Australia.

McArthur, W.M. & Bettenay, E. (1974) *Development and Distribution of Soils of the Swan Coastal Plain, Western Australia*. CSIRO, Australia.

McKane, R.B., Johnson, L.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E., Kielland, K., Kwiatkowski, B.L., Laundre, J.A. & Murray, G. (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature*, **415**, 68–71.

Meixner, R.E. & Singer, M.J. (1985) Phosphorus fractions from a chronosequence of alluvial soils, San Joaquin Valley, California. *Soil Science*, **139**, 37–46.

Morford, S.L., Houlton, B.Z. & Dahlgren, R.A. (2011) Increased forest ecosystem carbon and nitrogen storage from nitrogen rich bedrock. *Nature*, **477**, 78–81.

Murray-Wallace, C.V. & Kimber, R.W.L. (1989) Quaternary marine aminostratigraphy: Perth Basin, Western Australia. *Australian Journal of Earth Sciences*, **36**, 553–568.

Newsome, D. (2000) Origin of sandplains in Western Australia: a review of the debate and some recent findings. *Australian Journal of Earth Sciences*, **47**, 695–706.

Odum, E.P. (1969) The strategy of ecosystem development. *Science*, **164**, 262–270.

Olde Venterink, H. & Güsewell, S. (2010) Competitive interactions between two meadow grasses under nitrogen and phosphorus limitation. *Functional Ecology*, **24**, 877–886.

Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D.A. & Lambers, H. (2006) *Triticum aestivum* shows a greater biomass response to a supply of aluminium phosphate than *Lupinus albus*, despite releasing fewer carboxylates into the rhizosphere. *New Phytologist*, **169**, 515–524.

Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D.A. & Lambers, H. (2007) Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. *New Phytologist*, **173**, 181–190.

Peltzer, D.A., Wardle, D.A., Allison, V.J., Baisden, W.T., Bardgett, R.D., Chadwick, O.A. *et al.* (2010) Understanding ecosystem retrogression. *Ecological Monographs*, **80**, 509–529.

Pinheiro, J.C. & Bates, D.M. (2000) *Mixed-Effects Models in S and S-PLUS*. Springer, New York, USA.

Pinheiro, J., Bates, D. & DebRoy, S. (2010) *Nlme: Linear and Nonlinear Mixed Effects Models*. The Comprehensive R Archive Network (CRAN), Vienna, Austria.

Playford, P.E., Cockbain, A.E. & Low, G.H. (1976) *Geology of the Perth Basin, Western Australia*. Geological Survey, Western Australia, Perth, Australia.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Raghothama, K.G. (2000) Phosphorus acquisition; plant in the driver's seat! *Trends in Plant Science*, **5**, 412–413.

Richardson, S.J., Peltzer, D.A., Allen, R.B., McGlone, M.S. & Parfitt, R.L. (2004) Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia*, **139**, 267–276.

Sakaguchi, T., Nishizawa, N., Nakanishi, H., Yoshimura, E. & Mori, S. (1999) The role of potassium in the secretion of mugineic acids family phytosiderophores from iron-deficient barley roots. *Plant and Soil*, **215**, 221–227.

Santiago, L.S., Wright, S.J., Harms, K.E., Yavitt, J.B., Korine, C., Garcia, M.N. *et al.* (2012) Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. *Journal of Ecology*, **100**, 309–316.

Searle, D.J. & Woods, P.J. (1986) Detailed documentation in the Perth region, of a Holocene sea-level record, south-western Australia. *Quaternary Research*, **26**, 299–308.

Selmants, P.C. & Hart, S.C. (2010) Phosphorus and soil development: does the Walker and Syers model apply to semiarid ecosystems? *Ecology*, **91**, 474–484.

Semeniuk, V. & Glassford, D.K. (1988) Significance of aeolian limestone lenses in quartz sand formations: an interdigitation of coastal and continental facies, Perth Basin, southwestern Australia. *Sedimentary Geology*, **57**, 199–209.

Smith, S.E. & Read, D.J. (2008) *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, London, UK.

Tang, C., Robson, A., Longnecker, N. & Buirchell, B. (1995) The growth of *Lupinus* species on alkaline soils. *Australian Journal of Agricultural Research*, **46**, 255–268.

Tapsell, P., Newsome, D. & Bastian, L. (2003) Origin of yellow sand from Tamala Limestone on the Swan Coastal Plain, Western Australia. *Australian Journal of Earth Sciences*, **50**, 331–342.

Taylor, S.R. & McClennan, M. (1985) *The Continental Crust: Its Composition and Evolution*. Blackwell Scientific, Oxford, UK.

Thompson, C.H. (1981) Podzol chronosequences on coastal dunes of eastern Australia. *Nature*, **291**, 59–61.

Turner, B.L. (2008) Resource partitioning for soil phosphorus: a hypothesis. *Journal of Ecology*, **96**, 698–702.

Turner, B.L. & Romero, T.E. (2009) Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Science Society of America Journal*, **73**, 1972.

Van Duren, I.C. & Pegtel, D.M. (2000) Nutrient limitations in wet, drained and rewetted fen meadows: evaluation of methods and results. *Plant and Soil*, **220**, 35–47.

Vitousek, P.M. & Farrington, H. (1997) Nutrient limitation and soil development: experimental test of a biogeochemical theory. *Biogeochemistry*, **37**, 63–75.

Vitousek, P.M., Turner, D.R. & Kitayama, K. (1995) Foliar nutrients during long-term soil development in Hawaiian montane rain forest. *Ecology*, **76**, 712–720.

Vitousek, P.M., Walker, L.R., Whiteaker, L.D. & Matson, P.A. (1993) Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry*, **23**, 197–215.

Vitousek, P.M., Porder, S., Houlton, B.Z. & Chadwick, O.A. (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, **20**, 5–15.

Walker, T.W. & Syers, J.K. (1976) The fate of phosphorus during pedogenesis. *Geoderma*, **15**, 1–19.

Wardle, D.A., Walker, L.R. & Bardgett, R.D. (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. *Science*, **305**, 509–513.

Wardle, D.A., Bardgett, R.D., Walker, L.R., Peltzer, D.A. & Lagerstrom, A. (2008) The response of plant diversity to ecosystem retrogression: evidence from contrasting long-term chronosequences. *Oikos*, **117**, 93–103.

Wehmiller, J.F. & Miller, G.H. (2000) Aminostratigraphic dating methods in Quaternary geology. *Quaternary Geochronology: Methods and Applications* (eds J.S. Noller, J.M. Sowers & W.R. Lettis), pp. 187–222. American Geophysical Union, Washington DC, USA.

Wheeler, B.D., Shaw, S.C. & Cook, R.E.D. (1992) Phytometric assessment of the fertility of undrained rich-fen soils. *Journal of Applied Ecology*, **29**, 466–475.

Wright, S.J., Yavitt, J.B., Wurzburger, N., Turner, B.L., Tanner, E.V.J., Sayer, E.J., Santiago, L.S., Kaspari, M., Hedin, L.O., Harms, K.E., Garcia, M.N. & Corre, M.D. (2011) Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology*, **92**, 1616–1625.

Wyrwoll, K.-H. & King, P.D. (1984) A criticism of the proposed regional extent of Late Cenozoic arid zone advances into south-western Australia. *Catena*, **11**, 273–288.

Xu, G., Magen, H., Tarchitzky, J. & Kafkafi, U. (1999) Advances in chloride nutrition of plants. *Advances in Agronomy*, **68**, 97–150.

Zuur, A.F., Ieno, E.N., Walker, Neil.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, USA.

Received 28 November 2011; accepted 1 February 2012
Handling Editor: Rien Aerts

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Nutrients used in the different nutrient-addition treatments.

Table S2. Total amount of nutrients (mg kg^{-1} soil) supplied to plants during the entire growth period for the different nutrient addition treatments. Nutrients were supplied in six consecutive weekly doses.

Figure S1. Map of the study area showing site locations.

Figure S2. Root:shoot ratio responses of canola and wheat to nutrient-addition treatments in the different soils.

Figure S3. Growth and root:shoot ratio responses of white lupin to nutrient-addition treatments in the different soils.

Figure S4. Leaf greenness index measured on white lupin plants.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be reorganized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main article.

Our results provide strong support for the long-term ecosystem-development model, particularly with regard to the appearance of P limitation and associated declines in productivity. However, our study also shows that N cannot be assumed to invariably be the most important limiting nutrient in young soils, and it is unlikely to be the only limiting nutrient in calcareous soils. This south-western Australian long-term chronosequence provides an excellent opportunity to explore edaphic controls over plant species diversity.