Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence

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Summary

1. Long-term pedogenesis leads to important changes in the availability of soil nutrients, especially nitrogen (N) and phosphorus (P). Changes in the availability of micronutrients can also occur, but are less well understood. We explored whether changes in leaf nutrient concentrations and resorption were consistent with a shift from N to P limitation of plant productivity with soil age along a > 2-million-year dune chronosequence in south-western Australia. We also compared these traits among plants of contrasting nutrient-acquisition strategies, focusing on N, P and micronutrients.

2. The range in leaf [P] for individual species along the chronosequence was exceptionally large for both green (103–3000 μg P g^{-1}) and senesced (19–5600 μg P g^{-1}) leaves, almost equalling that found globally. From the youngest to the oldest soil, cover-weighted mean leaf [P] declined from 1840 to 228 μg P g^{-1}, while P-resorption efficiency increased from 0% to 79%. All species converged towards a highly conservative P-use strategy on the oldest soils.

3. Declines in cover-weighted mean leaf [N] with soil age were less strong than for leaf [P], ranging from 13.4 mg N g^{-1} on the youngest soil to 9.5 mg N g^{-1} on the oldest soil. However, mean leaf N-resorption efficiency was greatest (45%) on the youngest, N-poor soils. Leaf N:P ratio increased from 8 on the youngest soil to 42 on the oldest soil.

4. Leaf zinc (Zn) concentrations were low across all chronosequence stages, but mean Zn-resorption efficiency was greatest (55–74%) on the youngest calcareous dunes, reflecting low Zn availability at high pH.

5. N$_2$-fixing species had high leaf [N] compared with other species. Non-mycorrhizal species had very low leaf [P] and accumulated Mn across all soils. We surmise that this reflects Mn solubilization by organic acids released for P acquisition.

6. *Synthesis.* Our results show community-wide variation in leaf nutrient concentrations and resorption that is consistent with a shift from N to P limitation during long-term ecosystem development. High Zn resorption on young calcareous dunes supports the possibility of micronutrient co-limitation. High leaf [Mn] on older dunes suggests the importance of carboxylate release for P acquisition. Our results show a strong effect of soil nutrient availability on nutrient-use efficiency and reveal considerable differences among plants of contrasting nutrient-acquisition strategies.

Key-words: manganese accumulation, nitrogen, nutrient-resorption efficiency, nutrient-resorption proficiency, nutrient-use efficiency, phosphorus, plant–soil (below-ground) interactions, soil fertility gradient, zinc

Introduction

Leaf nutrient concentrations and resorption play a major role in determining leaf-level nutrient-use efficiency (NUE) – the amount of dry matter production per unit nutrient taken up (Small 1972; Vitousek 1982; Berendse & Aerts 1987; Killingbeck 1996; Aerts & Chapin 2000). Concentrations of nutrients in senesced leaves also strongly influence ecosystem nutrient cycling by affecting litter quality and thus litter decomposition rates (Crews *et al.* 1995; Aerts & Chapin 2000; Kitayama *et al.* 2004; Wardle *et al.* 2009). Moreover, leaf nutrient concentrations reflect the nature and strength of

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nutrient limitation in ecosystems (Black 1968; Grime 1979; Güsewell *et al.* 2005). The study of leaf nutrient concentrations and resorption in natural ecosystems can therefore indicate how individual plants cope with low nutrient availability and reveal feedbacks on nutrient cycling and plant productivity via litterfall (Small 1972; Boerner 1984; Aerts & Chapin 2000). This is particularly important and well understood for the cycling of nitrogen (N) and phosphorus (P), the two macronutrients that most often limit terrestrial plant productivity (Elser *et al.* 2007).

The relative importance of mature leaf nutrient concentration or resorption in maximizing leaf-level NUE can vary among nutrients. For example, N-use efficiency is thought to be most efficiently maximized by maintaining low leaf [N], whereas P-use efficiency is thought to be most efficiently maximized through a high P-resorption efficiency (Aerts & Chapin 2000). This difference in the factors contributing most to plant P-use efficiency vs. N-use efficiency is attributed to the greater capacity of plants to resorb P (upper limit ~90%) than N (upper limit ~80%), a difference that has significant consequences for plant NUE (Walbridge 1991; Aerts 1996; Killingbeck 1996; Aerts & Chapin 2000). As a result, different leaf traits would be expected to be favoured under N or P limitation of plant productivity.

Natural gradients of soil nutrient availability such as long-term soil chronosequences (Peltzer *et al.* 2010) can provide valuable model systems to study changes in leaf nutrient concentrations and resorption within and across plant species (Crews *et al.* 1995; Vitousek, Turner & Kitayama 1995; Richardson *et al.* 2004; Lagerström, Nilsson & Wardle 2013). Indeed, nutrient limitation of plant productivity typically shifts from N to P limitation as soils age along long-term soil chronosequences (Vitousek & Farrington 1997; Laliberté *et al.* 2012; Coomes *et al.* 2013). Such changes in the strength and type of nutrient limitation influences leaf nutrient concentrations and resorption and therefore leaf-level NUE (Killingbeck 1996; Richardson *et al.* 2004; Richardson, Allen & Doherty 2008; Hidaka & Kitayama 2011). Community-level changes in leaf nutrient concentration and resorption with respect to soil fertility are associated both with variation among species and with changes in species composition (Richardson *et al.* 2005; Wardle *et al.* 2009).

While a number of studies have explored how leaf nutrient concentrations and resorption vary with soil nutrient availability or among broad plant functional types (e.g. Aerts 1996; Kobe, Lepczyk & Iyer 2005; Vergutz *et al.* 2012), no study has yet examined how these traits vary among plants with different nutrient-acquisition strategies across broad nutrient-availability gradients such as long-term soil chronosequences. This is important because there can be significant differences in nutrient use among plants of contrasting nutrient-acquisition strategies (Lambers *et al.* 2010). For example, N₂-fixing species often show high leaf [N] and lower leaf N resorption (Vergutz *et al.* 2012), reflecting an 'N-demanding lifestyle' (McKey 1994). In addition, some non-mycorrhizal species from P-impoverished soils (e.g. Proteaceae in south-western Australia) show exceptionally high P-use efficiency by maintaining low leaf [P] and high P resorption (Denton *et al.* 2007). Interestingly, many of these non-mycorrhizal Proteaceae species also accumulate high levels of manganese (Mn) in their leaves (Jaffré 1979; Shane & Lambers 2005). This might be a side-effect of the large amounts of organic acids released from the roots of these plants, which not only mobilizes sorbed P (Lambers *et al.* 2006), but also increases Mn solubility (Godo & Reisenauer 1980; Jauregui & Reisenauer 1982). Therefore, reductions in soil P availability might lead not only to lower leaf [P] and higher P resorption, but also to higher leaf [Mn] for non-mycorrhizal species that release organic acids for P acquisition.

In this study, we determined leaf nutrient concentrations and resorption for plants of contrasting nutrient-acquisition strategies along the > 2-million-year Jurien Bay dune chronosequence in south-western Australia. This chronosequence shows the expected shift from N to P limitation (Laliberté *et al.* 2012). However, we focus not only on leaf N and P, but also consider other nutrients, since a previous study showed that potassium (K) or micronutrients may co-limit plant growth on young calcareous soils in this chronosequence (Laliberté *et al.* 2012, 2013; Uren & Parsons 2013). Importantly, one special characteristic of the Jurien Bay dune chronosequence is its location in a region of exceptionally high species diversity (Pate & Beard 1984; Myers *et al.* 2000; Hopper & Gioia 2004), with a high functional diversity of nutrient-acquisition strategies (Lamont 1982; Lambers *et al.* 2010). This provides an unparalleled opportunity to investigate changes in leaf nutrient concentrations and resorption in plants of different nutrient-acquisition strategies during long-term ecosystem development. The most abundant strategies along this dune chronosequence include associations with arbuscular mycorrhizal or ectomycorrhizal fungi, non-mycorrhizal strategies (e.g. cluster roots, dauciform roots and sand-binding roots; Shane, Dixon & Lambers 2005; Lambers *et al.* 2006; Smith, Hopper & Shane 2011), and species able to symbiotically fix atmospheric N₂.

We hypothesized that both soil age and nutrient-acquisition strategy would have a significant influence on leaf nutrient concentrations and resorption along the Jurien Bay dune chronosequence. Specifically, we predicted that leaf [P] would decrease sharply, and leaf P resorption increase, with decreasing P availability in older soils. On the other hand, we expected low leaf [N] and high N resorption on the youngest soils, where plant growth was shown previously to be N-limited (Laliberté *et al.* 2012). We also hypothesized that there would be a significant increase in leaf N : P ratios with increasing soil age, reflecting a shift from N to P limitation (Koerselman & Meuleman 1996; Güsewell 2004). With regard to nutrient-acquisition strategy, N₂-fixing species were expected to show higher leaf [N] and lower N resorption, whereas non-mycorrhizal species were expected to show low leaf [P] and high P resorption. Furthermore, we expected arbuscular mycorrhizal species to show higher leaf [P] and lower P resorption, with ectomycorrhizal species expected to show relatively lower leaf [P] and higher P resorption. Finally, we hypothesized that non-mycorrhizal plants would

show leaf Mn accumulation, reflecting their release of large amounts of organic acids to acquire P, and that such Mn accumulation would be most important on older soils where P is the key limiting nutrient. We also considered other micronutrients (i.e. Cu, Fe and Zn), because it had been previously suggested that micronutrients could co-limit plant growth on the youngest calcareous dunes (Laliberté *et al.* 2012). Overall, our study aimed to provide a comprehensive assessment of leaf nutrient use from plants of contrasting nutrient-acquisition strategies across an exceptionally broad gradient in soil nutrient availability in a global biodiversity hotspot (Laliberté *et al.* 2012).

Materials and methods

STUDY AREA AND STUDY SITE

The > 2-million-year Jurien Bay dune chronosequence is located in south-western Australia, approximately 200 km north of Perth. A detailed description of the dune chronosequence is presented in Laliberté *et al.* (2012). Essentially, the Jurien Bay dune chronosequence consists of a series of overlapping dune systems within ~10 km of the coast; the dunes were deposited during various periods of high sea level from the Early Pleistocene (and possibly Late Pliocene) to the present (Laliberté *et al.* 2012). The different dune systems increase in age with distance from the coast and have not been buried by younger sediments. As a result, they have been exposed to weathering since their deposition, thus creating a clear west–east soil age gradient. This soil age gradient is associated with large changes in soil nutrient availability and the type of nutrient limitation (Laliberté *et al.* 2012, 2013; Uren & Parsons 2013) that match expectations from the Walker & Syers (1976) model of soil development. Indeed, a previous nutrient-limitation bioassay showed that plant growth is N-limited on very young dunes [although potassium (K) or micronutrients may also play a role], co-limited by N, P and/or other nutrients on intermediate-aged dunes, and P-limited on old dunes (hundreds of thousands to millions of years old; Laliberté *et al.* 2012).

We used the same five chronosequence stages as studied in Laliberté *et al.* (2012), with the exception of stage 1, which in the present study represented young mobile dunes with established vegetation instead of unvegetated mobile dunes as used by Laliberté *et al.* (2012). The main geological and edaphic characteristics of these five chronosequence stages are summarized in Table 1.

The climate of the study area is Mediterranean, with hot, dry summers and cool, wet winters. Mean annual rainfall (1968–2013) is 538 mm, ~80% of which falls between May and September. Mean annual maximum temperature is 25 °C, with the warmest mean monthly maximum temperature being 31 °C (February) and the coolest 20 °C (July; Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/). In the 12 months prior to sampling (June 2011 to May 2012), the annual rainfall was 553 mm and the monthly maximum temperature was 26 °C (Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/). Fire is the main disturbance in the area, with typical fire-return intervals of < 30 years (Department of Conservation & Land Management 1995).

SITE SELECTION

For leaf sampling, we used 50 plots (10 × 10 m each) where vegetation had been characterized previously (G. Zemunik, unpublished

Table 1. Main features of the five chronosequence stages used in this study. Chronosequence stages, estimated soil ages, most likely (co-) limiting nutrient(s), and parent material are based on Laliberté *et al.* (2012). Names of dune systems are based on McArthur & Bettenay (1974). Geological formations are based on Playford, Cockbain & Low (1976)

Chronosequence stage	Dune system	Geological formation	Parent material	Estimated soil age (ka)	Most likely (co-) limiting nutrient(s)	Total N (g kg⁻¹)	Total P (mg kg⁻¹)	pH (CaCl₂)	CEC (cmol_c kg⁻¹)
1	Quindalup (young)	Safety Bay Sand	Calcareous sand	0.05 (Holocene)	N	0.51 ± 0.01	350.9 ± 2.4	8.15 ± 0.01	24.8 ± 1.5
2	Quindalup (medium)	Safety Bay Sand	Calcareous sand	1 (Holocene)	N, P and/or other nutrients	1.16 ± 0.01	432.2 ± 4.8	7.84 ± 0.01	12.9 ± 0.5
3	Quindalup (old)	Safety Bay Sand	Calcareous sand	6.7 (Holocene)	N, P and/or other nutrients	0.95 ± 0.05	285.8 ± 13.5	7.77 ± 0.01	12.4 ± 0.5
4	Spearwood (west)	Tamala Limestone	Calcareous sand	125 (Middle Pleistocene)	P	0.28 ± 0.01	20.3 ± 0.5	5.77 ± 0.03	3.8 ± 0.1
5	Bassendean	Bassendean Sand	Siliciclastic sand with smaller carbonate component	> 2000 (Early Pleistocene to Late Pliocene)	P	0.24 ± 0.01	6.6 ± 0.2	4.75 ± 0.03	2.4 ± 0.1

CEC, cation-exchange capacity.
Values for soil chemical characteristics are means ± standard errors (SE). $n = 70$.

data). In each plot, seven 2×2 m subplots were positioned randomly. All individual plants were counted and identified to species in each 2×2 m subplot. The percentage canopy cover of each species was also estimated (i.e. projection of live foliage on ground). Relative cover (%) of each species per plot was then estimated as a fraction of total canopy cover over the seven subplots.

Locations for the 10×10 m plots were selected using a random stratified sampling design. First, aerial photographs, maps (i.e. soil, geology and topography) and on-ground verification (e.g. using soil morphological characteristics) were used to define six broad regions corresponding to distinct dune systems and/or soil types. These six regions were spread over a ~450 km² (i.e. approximately 45 km north-south × 10 km east-west) coastal area from south of Jurien Bay to Green Head. Secondly, these regions were digitized as polygons in a geographical information system (Quantum GIS, http://qgis.org/). Thirdly, all vehicle-accessible roads and tracks from the general area were also digitised into the GIS. Finally, randomly-positioned points within each of the six distinct dune systems were generated in the GIS, constraining locations between 40 and 200 m from a road or track in order to facilitate access. Due to the relatively small sizes of vegetation patches within the youngest dune system (i.e. stage 1; young mobile dunes), locations were randomly selected without consideration to road or track proximity. Ten plots per dune system were selected for vegetation surveys, for a total of 60 plots. However, we only used five of these dune systems in this study (i.e. 50 plots total), excluding one of the two Spearwood dune subsystems used for vegetation surveys. The average closest distance between two neighbouring plots was 2.1 km. This was deemed large enough to minimize the potential for spatial autocorrelation to influence the results.

SPECIES SELECTION

Using the vegetation survey data (G. Zemunik, unpublished data), we ranked species in each of the five chronosequence stages from the most to the least abundant, based on canopy cover estimates. We then selected 5–7 species from each stage, targeting the most-abundant species for each of four contrasting nutrient-acquisition strategies: arbuscular mycorrhizal (AM), ectomycorrhizal (EM), N_2-fixing (NF) and non-mycorrhizal (NM). Ericoid mycorrhizal species were not considered because they were not among the most-abundant species. The nutrient-acquisition strategy of each species was identified based on available evidence in the literature and for most species was based on information known at the genus level. We note that N_2-fixing species are generally arbuscular and/or ectomycorrhizal, but we considered them as a separate group because they often show high leaf [N] (McKey 1994; Vergutz *et al.* 2012). Species were selected from the 10 most-abundant species per stage, with the exception of stage 4 where the 18 most-abundant species were considered. The selected species accounted for between 38% (stage 5) and 65% (stage 1) of the total canopy cover of each stage (Table 2). A total of 18 species were selected for leaf sampling across all five chronosequence stages (Table 2). The four main types of plant nutrient-acquisition strategies were further divided into seven specific nutrient-acquisition strategies to differentiate among mycorrhizal strategies (i.e. strictly arbuscular mycorrhizal, strictly ectomycorrhizal, facultative arbuscular/ectomycorrhizal) and three non-mycorrhizal strategies (e.g. cluster roots, dauciform roots and sand-binding roots; Shane, Dixon & Lambers 2005; Lambers *et al.* 2006; Smith, Hopper & Shane 2011) (Table 2). However, these specific strategies were not considered in the statistical analyses.

Table 2. Species sampled across the Jurien Bay chronosequence, showing each species, partial percentage cover per chronosequence stage, nutrient-acquisition strategy and specific nutrient-acquisition strategy (see Materials and methods for details)

Species	Family	Chronosequence stage 1	2	3	4	5	Nutrient-acquisition strategy	Specific nutrient-acquisition strategy
Scaevola crassifolia	Goodeniaceae	24.9 (10)					AM	AM
Spyridium globulosum	Rhamnaceae	9.7 (10)					EM	AM/EM
Olearia axillaris	Asteraceae	9.4 (9)					AM	AM
Hardenbergia comptoniana	Fabaceae	3.2 (5)					NF	NF
Conostylis candicans subsp. *calcicola*	Haemodoraceae	2.6 (9)	10.5 (10)	9.1 (10)			NM	Sand-binding
Acacia rostellifera	Fabaceae	15.5 (10)	6.7 (7)	2.9 (9)	2.1 (5)		NF	NF
Melaleuca systena	Myrtaceae		16.3 (10)	26.5 (10)	2.6 (10)		EM	EM
Acanthocarpus preissii	Asparagaceae		7.2 (10)	2.2 (9)			AM	AM
Lepidosperma squamatum	Cyperaceae		3.5 (8)	11.9 (9)			NM	Dauciform
Banksia leptophylla var. *melletica*	Proteaceae				15.1 (9)		NM	Cluster
Hibbertia hypericoides	Dilleniaceae				13.2 (8)	6.3 (9)	AM	AM
Acacia spathulifolia	Fabaceae				9.5 (5)		NF	NF
Banksia sessilis var. *cygnorum*	Proteaceae				2.7 (9)		NM	Cluster
Mesomelaena pseudostygia	Cyperaceae				1.3 (9)	3.7 (10)	NM	Dauciform
Banksia attenuata	Proteaceae					11.5 (9)	NM	Cluster
Jacksonia floribunda	Fabaceae					8.6 (9)	NF	NF
Banksia menziesii	Proteaceae					4.3 (9)	NM	Cluster
Melaleuca leuropoma	Myrtaceae					3.6 (9)	EM	EM
Total cover (%)		65.3	44.3	52.6	46.5	38.1		

AM, arbuscular mycorrhizal; EM, ectomycorrhizal; NM, non-mycorrhizal; NF, N_2-fixing.
The cumulative partial percentage cover accounted for is shown for each chronosequence stage in the last row. The number of samples taken for each species per stage is shown in brackets.

LEAF NUTRIENT CONCENTRATIONS

Leaf sampling

All leaf material was collected over a two-month period between late March and early May 2012, near the end of the dry summer season. In each of the 50 plots, only healthy mature individuals were selected for sampling. In general, mature and senesced leaves were sampled from one individual plant per species in each plot, and all leaves were bulked per species for each of the 50 plots. A species was considered absent from a plot if it could not be found within ~30 m of its centre. The number of individual collections (one collection = both mature and senesced leaves) per species in each chronosequence stage ranged from five to 10 (Table 2). In each case, representative samples of mature and senesced leaves were collected using nitrile gloves in order to minimize sample contamination. Leaves were not washed prior to nutrient analyses but we consider dust contamination to be highly unlikely, given the sandy nature of the soils.

Mature leaves were undamaged, fully expanded and exposed to full sunlight. In most cases, senesced leaves were collected directly from the plant by gently shaking the plant and collecting fallen leaves. Senesced leaves were easily distinguished from green leaves, since they were yellow or brown and detached easily from the plant. However, for a few species, it was not possible to collect senesced leaves from live plants, in which case senesced leaves were collected directly beneath the plant from recently fallen litter. In all cases, there was no visible degradation of senesced leaves collected from this litter, which had predominantly fallen during the summer and had not been exposed to any significant rain between litter fall and collection. Therefore, we assumed that losses of nutrients through leaching or decomposition were minimal, although some photodegradation (Austin & Vivanco 2006) might have occurred. A total of 508 leaf samples (mature and senesced) were collected for nutrient analyses.

Leaf nutrient analyses

Each leaf sample was oven-dried (70 °C, 48 h) and finely ground using a Teflon-coated stainless steel ball mill. A subsample was analysed for C and N concentrations using a continuous-flow system consisting of a SERCON 20–22 mass spectrometer connected with an automated nitrogen : carbon analyser (Sercon, Crewe, UK). A second subsample was acid-digested using concentrated HNO_3:$HClO_4$ (3:1) and analysed for Ca, Cu, Fe, K, Mg, Mn, Mo, P, S and Zn concentrations using inductively coupled plasma optical-emission spectrometry (ICP-OES; ChemCentre, Perth, WA, Australia). All digests were first analysed using a simultaneous Varian Vista Pro (Varian Australia Pty Ltd, Mulgrave, Victoria, Australia), radially configured ICP-OES equipment fitted with a charge-coupled device (CCD) detection system and an A.I. Scientific AIM-3600 auto-sampler. Samples with P concentrations close to minimum reporting limit were rerun on more sensitive axially-configured ICP-OES equipment.

SOIL SAMPLING AND NUTRIENT ANALYSES

Between 11 June and 16 June 2012, seven soil samples (one per 2 × 2 m subplot) were collected in each of the 60 10 × 10 m plots, for a total of 420 samples. Subsampling was done to obtain more accurate estimates of the mean. Samples were taken at 0–20 cm depth using a 50-mm diameter sand auger and were sieved (< 2 mm) to remove roots and other large organic debris. Samples were homogenised and air-dried prior to chemical analyses.

Total soil [P] was measured by ignition (550 °C, 1 h) and extraction in 1 M H_2SO_4, with phosphate detection by automated online neutralization and molybdate colourimetry using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO, USA). Total soil [N] was determined by combustion and gas chromatography on a Thermo Flash 1112 analyser (CE Elantech, Lakewood, NJ, USA). Total soil [Mn] was determined by digestion in concentrated nitric acid under pressure in PTFE vessels, followed by Mn detection by ICP-OES (Optima 7300DV; Perkin Elmer Inc, Wellesley, MA, USA). Soil pH was determined in a 1:2 soil-to-solution ratio in both water and 10 mM $CaCl_2$ using a glass electrode. Exchangeable cations were determined by extraction in 0.1 M $BaCl_2$ with detection by ICP-OES as above.

STATISTICAL ANALYSES

Nutrient-resorption efficiency (r) was calculated as the percentage reduction in leaf nutrient concentration from mature to senesced leaves (eqn 1):

$$r = 100 \frac{C_m - C_s}{C_m} \tag{1}$$

where C_m is the concentration of a particular nutrient in mature leaves and C_s is the nutrient concentration of senesced leaves (Aerts & Chapin 2000). Nutrient-resorption proficiency corresponds to the nutrient concentration in senesced leaves (Killingbeck 1996).

Differences in leaf nutrient concentration and resorption (proficiency and efficiency) between different chronosequence stages and nutrient-acquisition strategies (AM, EM, NF and NM), including the interaction between chronosequence stage and nutrient-acquisition strategy, were tested using general linear mixed-effect models (Pinheiro & Bates 2000). In these analyses, random intercepts per plot were specified, since more than one plant was sampled from the same plot. We also tested for differences in leaf nutrient concentration and resorption (proficiency and efficiency) at the community level, using generalised least squares models (Pinheiro & Bates 2000). These community-level analyses were done on cover-weighted leaf nutrient data for each plot in order to take into account changes in species relative abundances between plots (e.g. Richardson *et al.* 2004). When no plot-level cover estimate was available for a species, we used its dune-level (i.e. chronosequence stage) percentage cover estimate. For each model, residuals were visually inspected for heteroscedasticity. In the presence of heteroscedasticity, appropriate variance structures were specified if they significantly improved the models, as evaluated using likelihood ratio tests (Pinheiro & Bates 2000). Analyses were conducted in R, using the 'nlme' package (Pinheiro *et al.* 2012). The 'effects' package (Fox 2003) was used for further inspection of means and 95% confidence intervals (CI) to identify significant differences between chronosequence stages or nutrient-acquisition strategies.

Results

LEAF PHOSPHORUS CONCENTRATION AND RESORPTION

There was a broad range of mature leaf [P] across the chronosequence (Table S1 in Supporting Information), with mean values for individual species ranging from 103 µg P g^{-1} in *Mesomelaena pseudostygia* growing on the oldest soil (chronosequence stage 5) to 3000 µg P g^{-1} in *Olearia axillaris* on

the youngest soil (stage 1). Similarly, mean senesced leaf [P] varied almost 300-fold (Table S2), ranging from 19.4 µg P g^{-1} for *Banksia attenuata* in stage 5 to 5600 µg P g^{-1} for *Olearia axillaris* in stage 1. Finally, the range in P-resorption efficiency among individual species along the chronosequence was also high (Table S3), from –106% for *Olearia axillaris* in stage 1 (suggesting P accumulation in senesced leaves) to > 90% for *Banksia attenuata* in stage 5.

Differences in mature and senesced leaf [P] between nutrient-acquisition strategies depended on chronosequence stage (stage × strategy interaction; $P \leq 0.0001$; Fig. 1a,b). In short, differences in mature or senesced leaf [P] between nutrient-acquisition strategies were large for stage 1, but became smaller for older stages, as plants from all strategies converged to low leaf [P] (Fig. 1a), low senesced leaf [P] (Fig. 1b) and high P-resorption efficiency (Fig. 1c). Across all chronosequence stages, non-mycorrhizal species generally had the lowest mature leaf [P], while AM species had the highest, particularly in stages 1, 2 and 3 (Fig. 1a). Similar patterns were found for senesced leaf [P] (Fig. 1b). Phosphorus-resorption efficiency was significantly lower in AM species than in species from other strategies in stage 1 and also lower

in AM species than in NF and NM species in stage 5 (Fig. 1c).

At the community level (i.e. using cover-weighted values), both mature and senesced leaf [P] declined strongly with chronosequence stage, although the decline was more pronounced for senesced leaf [P] (Fig. 2a). As a result, leaf P-resorption efficiency increased sharply for older chronosequence stages, from no resorption in stage 1, to 79% resorption in stage 5 (Fig. 2b). In addition, the variance in mature and senesced leaf [P], as well as leaf P-resorption efficiency, decreased in older chronosequence stages (Fig. 2a,b), indicating functional convergence of co-occurring species. Declines in mature and senesced leaf [P] and increases in leaf P-resorption efficiency in progressively older chronosequence stages were linked to declining total soil [P] (Fig. 3a–c).

LEAF NITROGEN CONCENTRATION AND RESORPTION

Differences in mature and senesced leaf [N] between nutrient-acquisition strategies depended on chronosequence stage (stage × strategy interaction; $P \leq 0.0001$; Fig. 4a,b). Despite this interaction, mature and senesced leaf [N] was generally
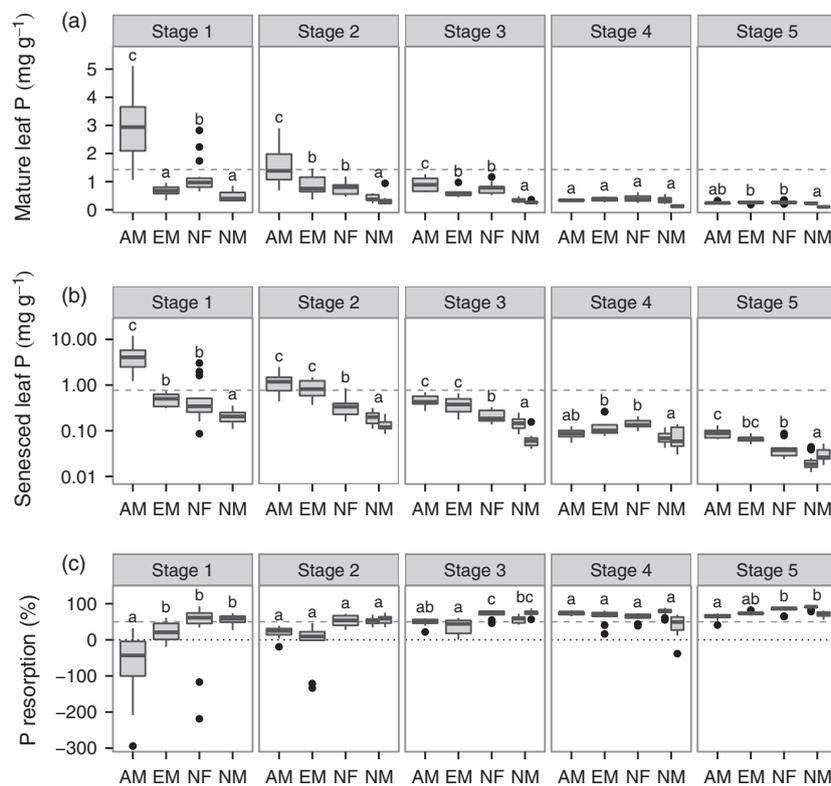

Fig. 1. Boxplots showing (a) mature leaf phosphorus concentrations ([P]), (b) senesced leaf [P] and (c) P-resorption efficiency (%) across the five chronosequence stages for plants of contrasting nutrient-acquisition strategies: AM (arbuscular mycorrhizal), EM (ectomycorrhizal), NF (N$_2$-fixing) and NM (non-mycorrhizal). The central vertical bar in each box shows the median, the box represents the interquartile range (IQR), the whiskers show the location of the most extreme data points that are still within a factor of 1.5 of the upper or lower quartiles, and the black points are values that fall outside the whiskers. Within each individual panel, different letters indicate significant differences between nutrient-acquisition strategies based on 95% confidence intervals from linear mixed-effect models. In (b), the y-axis is on a logarithmic scale. Specific non-mycorrhizal types in stages 2–5 are separated in different boxes. In stages 2 and 3, the left box represents the sand-binding strategy, whereas in stages 4 and 5, the left box represents the cluster-root strategy; in both cases the right box represents the dauciform strategy. Grey dashed lines indicate global averages for (a) mature leaf [P], (b) senesced leaf [P], and (c) P-resorption efficiency, uncorrected for leaf mass loss (Vergutz *et al.* 2012). In (c), a negative P-resorption efficiency indicates a higher leaf [P] in senesced leaves than in mature leaves.
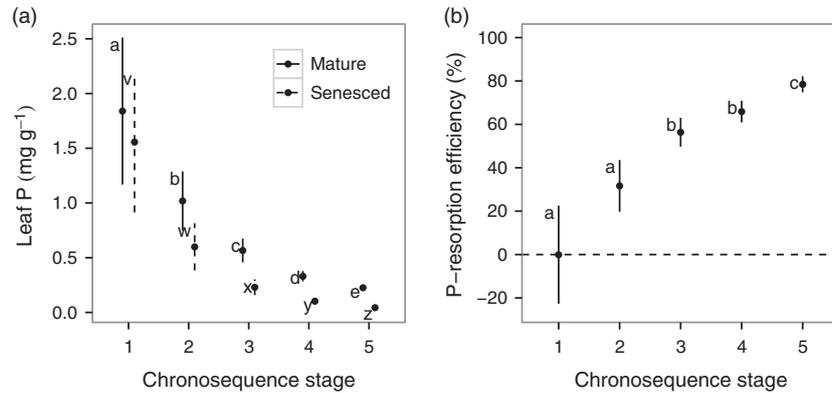
Fig. 2. Community-level, cover-weighted (a) mature and senesced leaf phosphorus (P) concentration ([P]) and (b) leaf P-resorption efficiency across the five chronosequence stages. Points indicate means and bar show 95% confidence intervals (CI) from linear mixed-effect models. Different letters indicate significant differences between chronosequence stages based on 95% CI. In panel (a) letters can only be compared with each other within a given leaf type (mature or senesced).
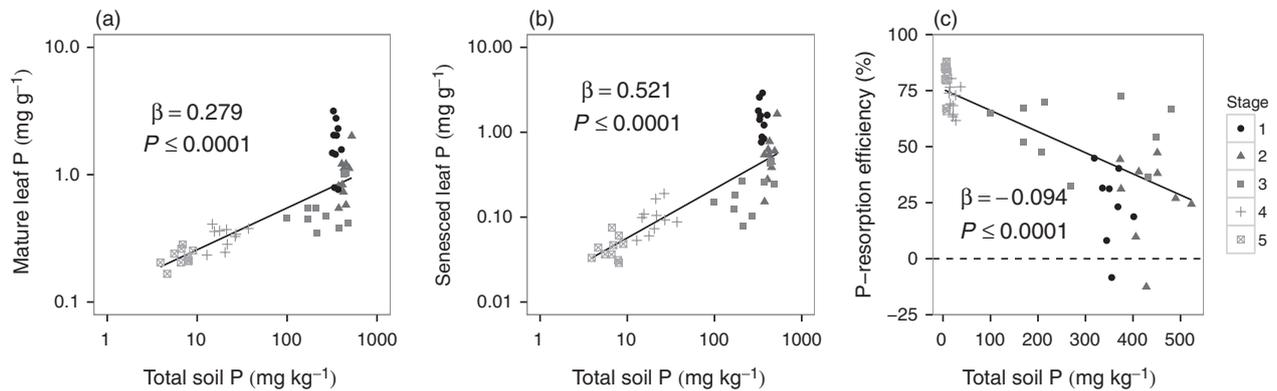

Fig. 3. Changes in community-level, cover-weighted (a) mature leaf phosphorus (P) concentration ([P]), (b) senesced leaf [P], and (c) leaf P-resorption efficiency with total soil [P]. Axes in panels (a, b) are on logarithmic scales. Black lines represent the lines of best fit derived from generalized least squares models. β = regression coefficients. In panels (a, b) generalized least square models were run on log-transformed leaf and soil [P] data.

greater in arbuscular mycorrhizal and/or N_2-fixing species compared with non-mycorrhizal and/or ectomycorrhizal species (Fig. 4a,b).

At the community level, cover-weighted mature leaf [N] was significantly higher in stages 1, 2 and 3 than in stages 4 and 5 (Fig. 5a). Importantly, senesced leaf [N] was significantly lower in stage 1 than in stages 2 or 3, and there were no differences in senesced leaf [N] between stages 1, 4 and 5 (Fig. 5a). Consequently, leaf N-resorption efficiency was greatest in stage 1, lowest in stages 2 and 3, and moderately high in stages 4 and 5 (Fig. 5b). As with [P], declines in mature and senesced leaf [N], and increases in leaf N-resorption efficiency, in older chronosequence stages were associated with lower total soil [N] (Fig. 6).

LEAF N : P RATIO

There was a strong increase in cover-weighted leaf N : P ratio with chronosequence stage, with mean leaf N : P ratio ranging from 8 in the youngest stage to 42 in the oldest stage

(Fig. 7). Based on suggested thresholds for N and P limitation (Koerselman & Meuleman 1996; Güsewell 2004), there was evidence for N limitation in stage 1, N/P co-limitation in stage 2, and increasingly severe P limitation in stages 3, 4 and 5 (Fig. 7).

LEAF MANGANESE CONCENTRATION AND RESORPTION

Differences in leaf [Mn] among nutrient-acquisition strategies varied among chronosequence stages (stage × strategy interaction; $P \leq 0.0001$). Despite this significant interaction, plants with non-mycorrhizal strategies showed consistently higher leaf [Mn] than plants with the other three nutrient-acquisition strategies in all chronosequence stages (Fig. 8). However, there were differences between specific non-mycorrhizal strategies within individual stages. In particular, the higher leaf [Mn] of the non-mycorrhizal strategy in stages 4 and 5 was due to cluster-rooted Proteaceae species (*Banskia attenuata* and *B. menziesii*), but not to the dauciform-rooted *Mesomelaena pseudostygia* (Fig. 8). At the community level,
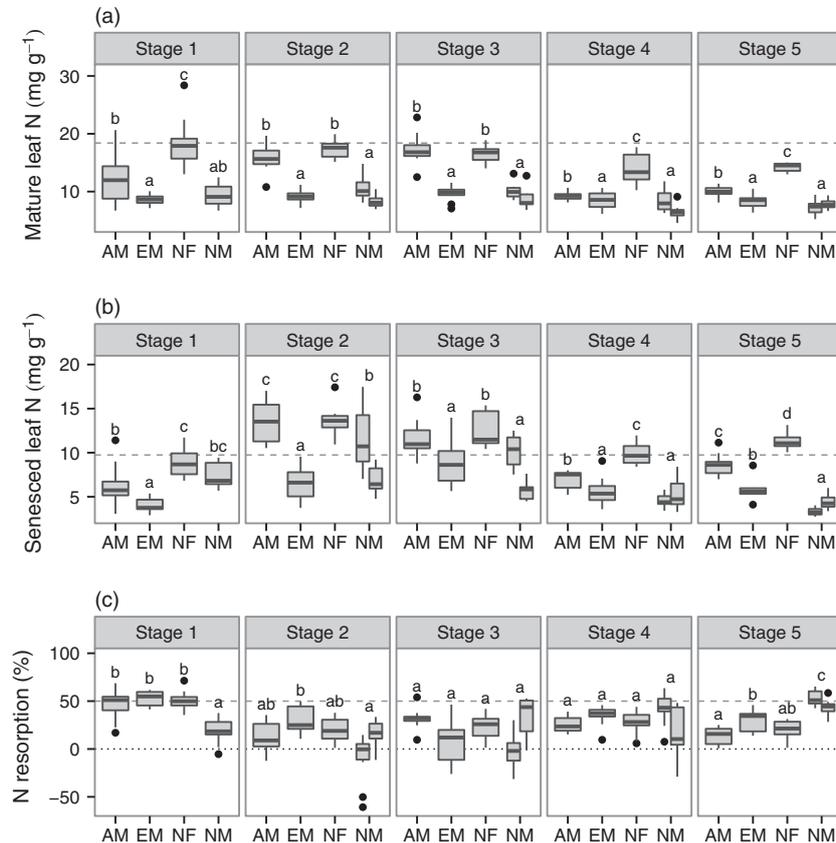
Fig. 4. Boxplots showing (a) mature leaf nitrogen (N) concentration ([N]), (b) senesced leaf [N] and (c) N-resorption efficiency (%) across the five chronosequence stages for plants of contrasting nutrient-acquisition strategies: AM (arbuscular mycorrhizal), EM (ectomycorrhizal), NF (N$_2$-fixing) and NM (non-mycorrhizal). The central vertical bar in each box shows the median, the box represents the interquartile range (IQR), the whiskers show the location of the most extreme data points that are still within a factor of 1.5 of the upper or lower quartiles, and the black points are values that fall outside the whiskers. Within each individual panel, different letters indicate significant differences between nutrient-acquisition strategies based on 95% confidence intervals from linear mixed-effect models. Specific non-mycorrhizal types in stages 2–5 are separated in different boxes. In stages 2 and 3, the left box represents the sand-binding strategy, whereas in stages 4 and 5, the left box represents the cluster-root strategy; in both cases the right box represents the dauciform strategy. Grey dashed lines indicate global averages for (a) mature leaf [N], (b) senesced leaf [N], and (c) N-resorption efficiency, uncorrected for leaf mass loss (Vergutz *et al.* 2012). In (c), a negative N-resorption efficiency indicates a higher leaf [N] in senesced leaves than in mature leaves.

cover-weighted mature leaf [Mn] generally increased in older chronosequence stages (Fig. 9). Specifically, leaf [Mn] was greater in stages 4 and 5 than in stage 2, and plants in stage 1 also showed lower leaf [Mn] than those in stage 5 (Fig. 9a). Leaf Mn-resorption efficiency was higher in stages 1, 2 and 3 (~25%) than in stages 4 and 5, where Mn was not resorbed during senescence (Fig. 9b).

A linear model showed that cover-weighted leaf [Mn] significantly increased with lower total soil [P] ($P = 0.0013$; Table S1 and Fig. S1), whereas soil pH and total soil [Mn] had no significant effects (Table S4). However, these results must be interpreted with caution, since total soil P and soil pH are significantly positively correlated ($r = 0.88$, $P < 0.0001$) along the dune sequence. An additional linear model using individual leaf [Mn] values for the three non-mycorrhizal species that occurred in at least two chronosequence stages (Table S5 and Fig. S2) showed that leaf [Mn] increased with decreasing total soil [P] ($P < 0.0001$) and soil pH ($P = 0.04$), whereas total soil [Mn] had no significant effect ($P = 0.07$).

OTHER NUTRIENTS

Other macronutrients (Ca, K, Mg and S; Fig. S3) and micronutrients (Cu, Fe, Mo and Zn; Fig. S4) showed no clear trends across the different chronosequence stages. However, mature leaf [Ca] and [S] concentrations were considerably higher in the N$_2$-fixing *Acacia* species (*A. rostellifera* and *A. spathulifolia*) than in other species. With the exception of K, all other macronutrients showed concentrations at or above those considered adequate for crop growth (Epstein & Bloom 2005; Fig. S3). Conversely, several micronutrients (i.e. Cu, Fe, Mo and Zn) showed concentrations at or below those considered adequate for crop growth (Fig. S4). In particular, leaf [Zn] and [Cu] were low in all plants across all soils (Fig. S4). In addition, cover-weighted Zn-resorption efficiency was much higher (55–74%) in stages 1, 2 and 3 than in stages 4 and 5 (< 12%; Fig. 9c). Iron- and Cu-resorption efficiencies, on the other hand, showed no clear patterns across chronosequence stages (Fig. S5).
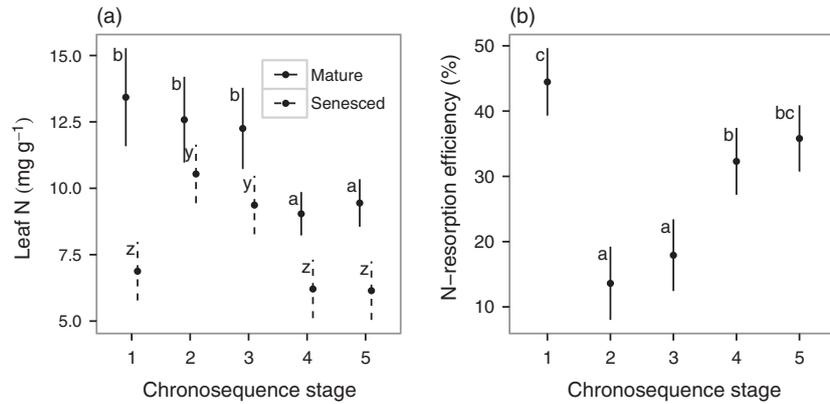
Fig. 5. Community-level, cover-weighted (a) mature and senesced leaf nitrogen (N) concentration ([N]) and (b) leaf N-resorption efficiency across the five chronosequence stages. Points indicate means and bar show 95% confidence intervals (CI) from linear mixed-effect models. Different letters indicate significant differences between chronosequence stages based on 95% CI. In panel (a) letters can only be compared with each other within a given leaf type (mature or senesced).
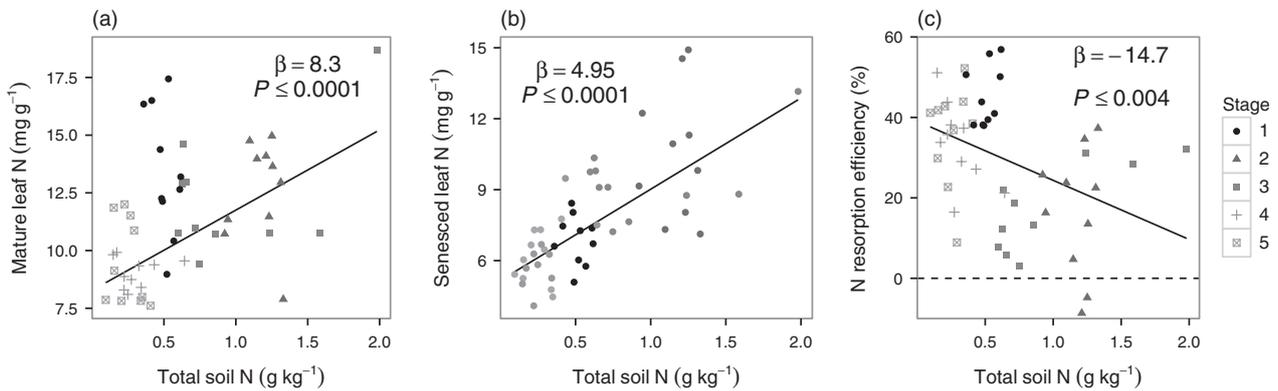

Fig. 6. Changes in community-level, cover-weighted (a) mature leaf nitrogen (N) concentration ([N]), (b) senesced leaf [N], and (c) leaf N-resorption efficiency with total soil [N]. Black lines represent the lines of best fit derived from generalized least squares models. β = regression coefficients.
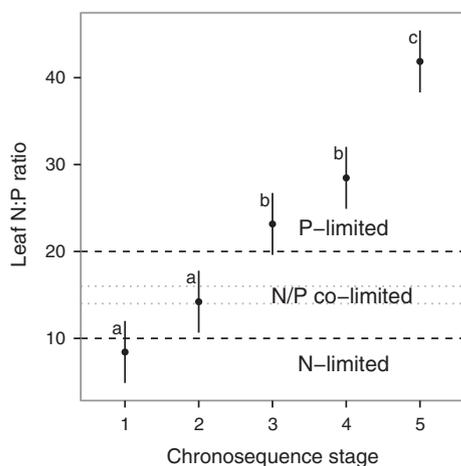

Fig. 7. Community-level, cover-weighted nitrogen (N): phosphorus (P) ratio of mature leaves across the five chronosequence stages. Points indicate means and bars show 95% confidence intervals (CI). $n = 10$. Different letters indicate significant differences between chronosequence stages based on 95% CI. Black dashed lines indicate thresholds for N or P limitation, following Güsewell (2004). Grey dotted lines indicate thresholds for N or P limitation based on Koerselman & Meuleman (1996).

Discussion

The results of our leaf nutrient survey along the Jurien Bay dune chronosequence provide strong support for the Walker & Syers (1976) pedogenesis model. Leaf nutrient concentrations and resorption, as well as leaf N : P ratios, indicated a clear shift from N to P limitation during long-term ecosystem development (Vitousek & Farrington 1997; Peltzer *et al.* 2010; Laliberté *et al.* 2012). Depletion-driven losses of soil P along the chronosequence were associated with marked declines in mature leaf [P] and strong increases in P-resorption efficiency and proficiency on older soils. Our study also showed that the range of individual leaf [P] and P-resorption values along the Jurien Bay chronosequence was exceptionally large, almost equalling the range of values recorded for natural systems world-wide (Lambers *et al.* 2011; Vergutz *et al.* 2012). In contrast, variation in mature leaf [N] and N resorption along the chronosequence was modest. There was also significant variation in leaf [P] and [N] among nutrient-acquisition strategies, with a convergence in all strategies towards high P-use efficiency on older soils, via both improved P resorption and reduced leaf [P]. In addition, our
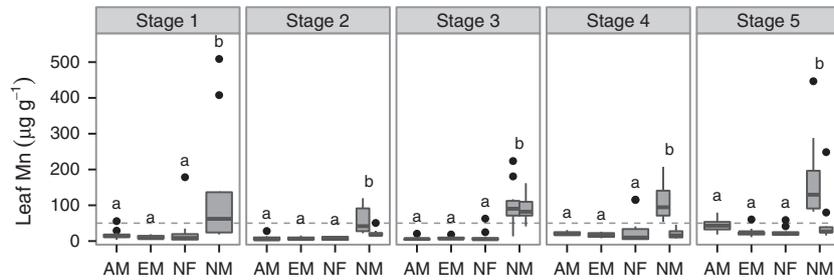
Fig. 8. Manganese (Mn) concentrations ([Mn]) in mature leaves across the five chronosequence stages for plants of contrasting nutrient-acquisition strategies: AM (arbuscular mycorrhizal), EM (ectomycorrhizal), NF (N_2-fixing) and NM (non-mycorrhizal). The central vertical bar in each box shows the median, the box represents the interquartile range (IQR), the whiskers show the location of the most extreme data points that are still within a factor of 1.5 of the upper or lower quartiles, and the black points are values that fall outside the whiskers. For each individual panel, different letters indicate significant differences between nutrient-acquisition strategies based on 95% confidence intervals. Specific non-mycorrhizal types in stages 2–5 are separated in different boxes. In stages 2 and 3, the left box represents the sand-binding strategy, whereas in stages 4 and 5, the left box represents the cluster-root strategy; in both cases the right box represents the dauciform strategy. The dashed grey line represents the mature leaf [Mn] considered adequate for crop growth (Epstein & Bloom 2005).
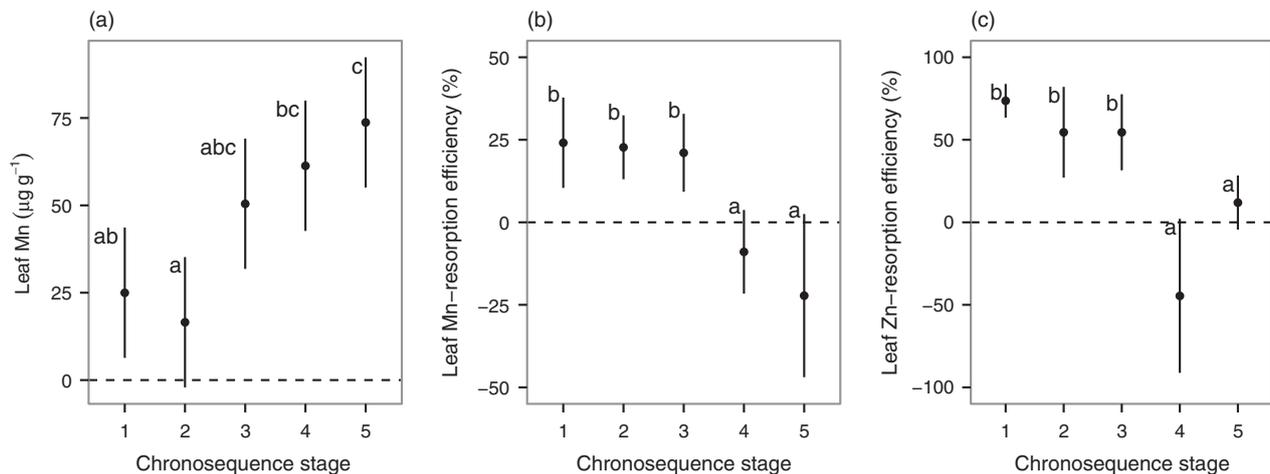

Fig. 9. Community-level, cover-weighted (a) mature leaf manganese (Mn) concentrations ([Mn]), (b) Mn-resorption efficiency, and (c) Zinc-resorption efficiency across the five chronosequence stages. Points indicate means and bars show 95% confidence intervals (CI). $n = 10$. Different letters indicate significant differences between chronosequence stages based on 95% CI.

results also highlighted important changes in micronutrients along the chronosequence. In particular, non-mycorrhizal species showed higher [Mn] in their leaves compared with all other strategies. We surmise that elevated leaf [Mn] is associated with plants releasing carboxylates to improve P acquisition. Finally, greater leaf Zn-resorption efficiency on the youngest calcareous dunes might reflect micronutrient co-limitation at high pH.

EXTREME RANGE IN LEAF PHOSPHORUS CONCENTRATION AND RESORPTION

A striking pattern observed in our study was the exceptionally strong decline in leaf [P] and associated increases in P resorption with increasing soil age, both of which were far greater than those reported for other well-studied chronosequences. Indeed, mature leaf [P] was > 8 times lower for plants on the oldest soils than for plants on the youngest soils, while senesced leaf [P] was > 30 times lower. In contrast, the Franz

Josef post-glacial chronosequence in New Zealand (Richardson *et al.* 2004) shows only a threefold difference in mean leaf [P] and a fivefold difference in P-resorption proficiency, between the 280-year (where leaf [P] was greatest) and the oldest (120 000-year, where leaf [P] was lowest) sites.

The high variation in leaf [P] and leaf P resorption along the Jurien Bay dune chronosequence is accounted for by two factors. First, total soil [P] varies markedly along the Jurien Bay chronosequence, with total soil [P] in surface soil ranging from 350 mg P kg^{-1} in the youngest soils to 7 mg P kg^{-1} in the oldest soils. This is attributed to the fact that the chronosequence covers a long period of soil development, from present day to Early Pleistocene, and possibly extending to the Late Pliocene (Hewgill *et al.* 1983; Kendrick, Wyrwoll & Szabo 1991; Hearty & O'Leary 2008; Laliberté *et al.* 2012, 2013). As a result, there has been a long period for depletion-driven P losses to occur.

The second factor accounting for the strong decline in leaf [P] along the Jurien Bay chronosequence is that its species

pool is much greater than that found on any other well-studied chronosequence. This maximizes the potential for variation in nutrient-use efficiency among species, which in turn maximizes variation in community-level leaf traits among chronosequence stages if species turnover between these stages is high (Vitousek 2004; Wardle *et al.* 2009). South-western Australia is unique in that both its climate and land-forms have been relatively stable over extremely long periods, and it hosts a very species-rich and mostly endemic flora that has persisted and evolved on ancient, strongly weathered, P-impoverished soils (Pate & Beard 1984; Hopper & Gioia 2004). We surmise that the large variation in leaf [P] and P resorption along the Jurien Bay dune chronosequence is due to the high species turnover across the different dune systems (Griffin & Hopkins 1990), which reflects a high degree of edaphic specialization of an ancient flora (Lambers *et al.* 2010, 2013). This is in marked contrast to the Hawaiian chronosequence, where all of the chronosequence stages are dominated by a single tree species, *Metrosideros polymorpha* (Vitousek 2004). The Jurien Bay chronosequence thus highlights the importance of a diverse species pool for maximizing variation in community-level leaf traits along soil nutrient-availability gradients.

We found high [P] in senesced leaves (sometimes higher than in mature leaves) of arbuscular mycorrhizal species on the youngest soil, where plant productivity is most likely limited by N (Laliberté *et al.* 2012). This may be explained by two factors. First, variability in leaf [P] among years might result in senesced leaves from previous years showing higher [P] (Killingbeck 1996; Aerts & Chapin 2000). Secondly, senesced leaves still attached to stems might lose some carbon through photodegradation in the sunny Mediterranean climate (Austin & Vivanco 2006), thus concentrating nutrients. As we did not consider leaf mass loss during senescence in our leaf nutrient-resorption measurements, this may have led to under-estimations of actual nutrient-resorption efficiency, possibly by as much as 10% (Vergutz *et al.* 2012). However, such potential under-estimation of nutrient-resorption efficiency would not alter the strong pattern of increasing leaf P-resorption efficiency along the Jurien Bay chronosequence.

DIFFERENCES IN PHOSPHORUS AND NITROGEN CONCENTRATIONS AMONG NUTRIENT-ACQUISITION STRATEGIES

Our study shows that along the Jurien Bay chronosequence, leaf [P] differs most strongly among plants of contrasting nutrient-acquisition strategies on the younger, more fertile soils. As soils age and become increasingly infertile, differences in leaf [P] among plants of different strategies are reduced. This reflects a convergence towards a highly conservative P-use strategy that maximizes P-use efficiency by maintaining low leaf [P] and high P resorption. Such functional convergence in leaf traits related to P-use efficiency on older soils has also been observed along the Franz Josef post-glacial chronosequence (Mason *et al.* 2012). Yet, despite this functional convergence in leaf [P] and P resorption on the

oldest, severely P-impoverished soils in the present study, non-mycorrhizal species (e.g. cluster-rooted Proteaceae) had lower leaf [P] than ectomycorrhizal or N_2-fixing plants and showed the highest P resorption of all strategies on these soils, indicative of a high P-use efficiency. This was expected since Proteaceae species from south-western Australia are renowned for both their exceptionally efficient acquisition and use of P (Lambers *et al.* 2006, 2012; Denton *et al.* 2007). Nevertheless, we also found that non-mycorrhizal species showed lower leaf [P] and higher leaf P resorption than species with other nutrient-acquisition strategies in most of the soils, even on the younger soils, which are not associated with P limitation (Laliberté *et al.* 2012). Therefore, non-mycorrhizal species in this region overall show a more conservative P-use strategy than plants of other strategies, regardless of soil P availability.

On younger soils, arbuscular mycorrhizal species had higher leaf [P] and lower P resorption than plants of other nutrient-acquisition strategies. A conceptual model of changes in the relative importance of nutrient-acquisition strategies with soil age proposed that the arbuscular mycorrhizal strategy is most important in relatively young soils (Lambers *et al.* 2008). Arbuscular mycorrhizas promote plant P acquisition by increasing the volume of soil explored, rather than via chemical alteration of the mycorhizosphere (Lambers, Chapin & Pons 2008; Smith & Read 2008). This strategy is particularly successful when P availability is relatively high, as it is in the studied younger soils (Laliberté *et al.* 2012). This might explain the higher leaf [P] of arbuscular mycorrhizal species on young soils.

Another notable difference in leaf nutrient concentration among nutrient-acquisition strategies was the higher leaf [N] of N_2-fixing species and to a lesser extent arbuscular mycorrhizal species, in most chronosequence stages. The higher leaf [N] of N_2-fixing species is a pattern that is found globally (Vergutz *et al.* 2012) and can be attributed to their N_2-fixation ability and/or 'N-demanding lifestyle' (McKey 1994). On the other hand, the higher leaf [N] of arbuscular mycorrhizal plants is more difficult to interpret. One possibility is that arbuscular mycorrhizal fungi might promote the uptake of ammonium (Johansen, Jakobsen & Jensen 1993; Tobar, Azcón & Barea 1994), which is less mobile than nitrate (Lambers, Chapin & Pons 2008).

MANGANESE ACCUMULATION IN NON-MYCORRHIZAL PLANTS

Species with different types of non-mycorrhizal strategies (i.e. cluster roots, dauciform roots and sand-binding roots) showed higher leaf [Mn] compared with species utilizing other nutrient-acquisition strategies, regardless of soil age. To our knowledge, this is the first study to show that Mn accumulation is consistent for a range of naturally occurring non-mycorrhizal strategies across soils of contrasting fertility. Manganese accumulation in leaves has previously been reported for Proteaceae species (with cluster roots) in New Caledonia (Jaffré 1979) and for *Hakea prostrata* (Proteaceae)

in Western Australia (Shane & Lambers 2005). It has been shown for *H. prostrata* that Mn accumulation is positively correlated with the proportion of roots invested in cluster roots and hence might be accounted for by increased carboxylate and proton release (Shane & Lambers 2005). For example, malate reduces MnO_2 and releases Mn^{2+} in the soil solution in both acidic and alkaline soils (Jauregui & Reisenauer 1982). Therefore, leaf Mn accumulation might act as an easily measurable proxy for carboxylate release, although further research is required to investigate this possible mechanism.

Interestingly, we found that, on average, species of all nutrient-acquisition strategies showed a gradual increase in leaf [Mn] with increasing soil age. This increase in leaf [Mn] was most strongly related to lower total soil [P]. The general increase in leaf [Mn] with lower soil [P] might be indirectly due to non-mycorrhizal plants mobilizing Mn through the exudation of large amounts of carboxylates and protons in their rhizosphere, which might facilitate Mn uptake by neighbouring plants of different nutrient-acquisition strategies. Such facilitation of Mn uptake of mycorrhizal plants by cluster-rooted non-mycorrhizal plants has been found between crop species (Gardner & Boundy 1983) as well as native south-western Australian species (Muler *et al.* 2013). Alternatively, it might also be due to increasing soil acidity in older soils across the chronosequence (Laliberté *et al.* 2012), thereby increasing the solubility of Mn (Lambers, Chapin & Pons 2008).

LEAF NITROGEN RESORPTION INCREASES NITROGEN-USE EFFICIENCY ON YOUNG SOILS

Variation in leaf [N] was modest along the Jurien Bay chronosequence compared with variation in leaf [P]. Leaf [N] was lowest in the two oldest chronosequence stages. This was expected based on previous results showing that these soils had low [N] (Laliberté *et al.* 2012), possibly because low [P] in these older soils constrains N_2 fixation (Vitousek *et al.* 2002) and/or does not favour N retention when P, rather than N, limits productivity. Leaf [N] also tends to be lower when leaf [P] is low and N : P ratios are high (Lambers *et al.* 2010). This might be partly accounted for by 'dilution' due to a high leaf mass per unit area, but plants also down-regulate their rate of N uptake and assimilation when P is in short supply (Rufty *et al.* 1993; Gniazdowska & Rychter 2000; De Groot *et al.* 2003). Lower leaf [N] (and hence lower leaf protein) may actually contribute to an increase in P-use efficiency, by allowing lower levels of ribosomal RNA, a major fraction of leaf P (Veneklaas *et al.* 2012). Indeed, ribosomal RNA is associated with net protein synthesis and protein turnover.

A more surprising result was the comparatively high leaf [N] on the youngest soil, despite this soil having low total and 'plant-available' [N] and being associated with N limitation of plant growth (Laliberté *et al.* 2012). Aerts & Chapin (2000) showed via a theoretical model that leaf-level N-use efficiency should be maximized by either maintaining leaf [N] < 15 mg g^{-1} or having N-resorption efficiencies > 75%, but

concluded through empirical analysis that the synthesis of leaves with low [N] was the most important trait to maximize leaf-level N-use efficiency. In contrast, our results show that N limitation on the youngest soil of the Jurien Bay chronosequence leads to higher N resorption, but not to lower mature leaf [N]. On the other hand, N-resorption efficiency was lower in stages 2 and 3, before increasing again in stages 4 and 5, while leaf [N] decreased consistently across this gradient. This change corresponds with the reduced soil [N] in stages 4 and 5, even though P is the key limiting nutrient in these older stages.

The reasons underlying the apparent lack of a selection pressure to maintain low leaf [N] on the youngest, N-poor soil remain unclear. Instead, our results suggest that plants growing on these young N-poor soils maximize N-use efficiency by increasing N-resorption efficiency. Still, we note that even though the N-resorption efficiency was greatest on the youngest soil (i.e. 45%), this value is not particularly high compared with the global average N-resorption efficiency of 50% (Aerts & Chapin 2000), or a more recent estimate of 62% that takes into account leaf mass loss during senescence (Vergutz *et al.* 2012).

LEAF N : P RATIO AND SHIFTS FROM N TO P LIMITATION

We found strong increases in leaf N : P ratios with increasing soil age along the chronosequence, from 8 on the youngest soil to 42 on the oldest. This increase in leaf N : P ratio was primarily linked to strong reductions in leaf [P] on older soils. In an analysis of 40 fertilization studies of European wetland ecosystems, Koerselman & Meuleman (1996) found that N : P ratios < 14 and > 16 were indicative of N or P limitation, respectively. Subsequently, Güsewell (2004) suggested more conservative N : P ratio threshold values of < 10 for N limitation and > 20 for P limitation. Based on these estimates, our results point to shifts from N limitation on the youngest soils (stage 1) to N/P co-limitation during stage 2 (and perhaps stage 3), and finally to P limitation on the oldest soils (stages 4 and 5).

The usefulness of N : P ratios to identify N or P limitation has been criticized (Craine, Morrow & Stock 2008), especially when leaves of different species are compared (such as in our study). However, results from a previous nutrient-addition glasshouse experiment using crop species as phytometers and soils collected along the Jurien Bay dune chronosequence (Laliberté *et al.* 2012) support such a shift from N to P limitation. In that study, the growth of crop plants growing on field-collected soils was N-limited (although K and micronutrients might also be important) on the youngest soil (stage 1), stages 2 and 3 were associated with co-limitation by N, P and/or other nutrients, whereas P limitation was found on the oldest soils (stages 4 and 5). Together, results from our present study and this previous glasshouse experiment (Laliberté *et al.* 2012) provide two independent lines of evidence that productivity shifts from being N-limited to P-limited along the Jurien Bay dune chronosequence. Future field fertilization

experiments (Specht, Connor & Clifford 1977; e.g. Vitousek & Farrington 1997) could help to test this hypothesis.

Even though the youngest chronosequence stage in our study contained shrubs capable of N_2-fixation with moderately high leaf N : P ratios (e.g. 14 for *Acacia rostellifera*, 19 for *Hardenbergia comptoniana*), we found a low cover-weighted N : P ratio that indicated N limitation. This is because two abundant arbuscular mycorrhizal species (*Scaevola crassifolia* and *Olearia axillaris*) growing on these youngest soils had high leaf [P], but only average leaf [N] and hence low N : P ratios (4 and 5, respectively). Factors explaining the success of these two species, which are not known to form associations with N_2-fixing bacteria, on soils with such low total [N] deserve further attention. In particular, a potential role for N_2-fixing bacterial endophytes (e.g. Oliveira *et al.* 2002; Bal *et al.* 2012; Taulé *et al.* 2012) in these species would be worth exploring.

OTHER NUTRIENTS

Despite the valuable inferences we can draw from N : P ratios with respect to nutrient limitation across soil fertility gradients, it is also essential to consider the importance of other macronutrients and micronutrients, and the roles they might play in limiting plant growth. Unlike some nutrients (e.g. Ca, S and Mg) that were found in leaves at concentrations considered to be adequate for crop plants (Epstein & Bloom 2005), most other nutrients (e.g. Cu, Fe, K, Mo and Zn) were found at concentrations below these values. While comparisons of leaf nutrient concentrations between crop and native plants can be problematic, K and micronutrients deserve further attention as potential drivers of primary productivity and plant species distributions along the Jurien Bay dune chronosequence, even though the key limiting nutrients appear to be N or P along that sequence (Laliberté *et al.* 2012). In particular, our results suggest that Zn may be limiting on the younger calcareous dunes (Laliberté *et al.* 2013; Uren & Parsons 2013), since mean Zn-resorption efficiency was significantly higher on those dunes than on older ones. The high leaf [Ca] and [S] in all *Acacia* species along the first four chronosequence stages is presumably associated with the biogenic formation of calcium sulphate biominerals, as observed on four *Acacia* species occurring in the Great Sandy Desert (He *et al.* 2012).

Conclusions

Our study shows that the strong gradient in N and P availability along the Jurien Bay dune chronosequence has a major influence on key plant traits related to nutrient-acquisition strategy and leaf-level nutrient-use efficiency. Depletion-driven losses of soil P during long-term ecosystem development lead to strong declines in leaf [P], coupled with marked increases in P resorption, measured as both proficiency and efficiency. On the other hand, selection for higher N-use efficiency on the N-poor youngest soils appears to operate via higher N-resorption proficiency and efficiency, but not via

lower leaf [N]. The fact that the clear patterns of leaf [N] and [P] with soil age obtained in the present study are consistent with expectations from long-term soil and ecosystem development (Vitousek & Farrington 1997; Richardson *et al.* 2004; Peltzer *et al.* 2010), whereas no clear patterns with soil age were found for most other nutrients and provide further evidence that productivity shifts from being N- to P-limited along the Jurien Bay dune chronosequence (Laliberté *et al.* 2012). However, our study also showed that there may be a selective pressure to remobilize leaf Zn during leaf senescence on young calcareous dunes (Laliberté *et al.* 2013; Uren & Parsons 2013), given that Zn is poorly available at high pH.

Another key result from our study was the fact that non-mycorrhizal plants consistently showed higher leaf [Mn] than plants with other nutrient-acquisition strategies. Leaf Mn accumulation in non-mycorrhizal plants probably reflects a reliance on carboxylate release for P acquisition, which mobilizes both P and Mn.

Overall, the present study highlights that the Jurien Bay dune chronosequence provides a strong nutrient-availability gradient that supports a diverse flora exhibiting an exceptionally high degree of functional variation in traits related to nutrient acquisition and use. As such, it provides excellent opportunities for future comparative ecophysiological studies, particularly with regard to N and P nutrition and use among species of contrasting nutrient-acquisition strategies.

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Supporting Information

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

Table S1. Nutrient concentrations of mature leaves for individual species with increasing chronosequence stage.

Table S2. Nutrient concentrations of senesced leaves for individual species with increasing chronosequence stage.

Table S3. Leaf nutrient-resorption efficiency (%) for individual species with increasing chronosequence stage.

Table S4. Results of linear mixed-effect model testing the effect of soil pH, total phosphorus (P) and total manganese (Mn) concentrations on (log-transformed) cover-weighted mature leaf [Mn].

Table S5. Results of linear mixed-effect model testing the effect of soil pH, total phosphorus (P) and total manganese (Mn) concentrations on (log-transformed) mature leaf [Mn] of the three non-mycorrhizal species that occurred across at least two chronosequence stages.

Figure S1. Effect of total soil phosphorus (P) concentration on cover-weighted leaf manganese (Mn) concentration.

Figure S2. Effect of total soil phosphorus (P) concentration on mature leaf manganese (Mn) concentration for the three non-mycorrhizal species that occurred across at least two chronosequence stages.

Figure S3. Boxplots showing mature leaf concentrations of different macronutrients (calcium [Ca], sulfur [S], magnesium [Mg] and potassium [K]) across the five chronosequence stages for plants of contrasting nutrient-acquisition strategies: AM, arbuscular mycorrhizal; EM, ectomycorrhizal; NF, N₂-fixing; and NM, non-mycorrhizal.

Figure S4. Boxplots showing mature leaf concentrations of different micronutrients (iron [Fe], zinc [Zn], copper [Cu] and molybdenum [Mo]) across the five chronosequence stages for plants of contrasting nutrient-acquisition strategies: AM, arbuscular mycorrhizal; EM, ectomycorrhizal; NF, N₂-fixing; and NM, non-mycorrhizal.

Figure S5. Community-level, cover-weighted (a) leaf iron (Fe) and (b) copper (Cu) resorption efficiencies across the five chronosequence stages.