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► To cite this version:

Felix de Tombeur, Etienne Laliberté, Hans Lambers, Michel-Pierre Faucon, Graham Zemunik, et al.. A shift from phenol to silica-based leaf defenses during long-term soil and ecosystem development. Ecology Letters, 2021, 24 (5), pp.984-995. 10.1111/ele.13713 . hal-03257100

HAL Id: hal-03257100

<https://hal.science/hal-03257100>

Submitted on 10 Jun 2021

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A shift from phenol to silica-based leaf defenses during long-term soil and ecosystem development

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Statement of authorship: All the authors conceived the ideas and designed methodology; FdT and GZ collected the samples; FdT analyzed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility statement: The data are available via the Dryad Digital Repository (<https://datadryad.org/stash/share/dRsI-z44EH1pPn-zuaujFqTuypkSqcXMZ-lvdMAp4Ac>)

Article type: Letters/Ideas & Perspectives

Running title: Leaf defenses during ecosystem development

Keywords: plant defense strategies ; silica-based defense ; plant silicon ; plant phenols ; resource availability hypothesis ; soil fertility gradient ; plant-herbivore interactions ; phenylalanine; resource limitation ; tradeoffs

Number of words in the Abstract (148), in the Main Text (5000)

Number of References: 99 (+ 3 references in the Supplementary Information)

Number of Figures and Tables: 6

Abstract

The resource availability hypothesis predicts that plants adapted to infertile soils have high levels of anti-herbivore leaf defenses. This hypothesis has been mostly explored for secondary metabolites such as phenolics, while it remains underexplored for silica-based defenses. We determined leaf concentrations of total phenols and silicon (Si) in plants growing along the 2-million-year Jurien Bay chronosequence, exhibiting an extreme gradient of soil fertility. We found that nitrogen (N) limitation on young soils led to a greater expression of phenol-based defenses, whereas old, phosphorus (P)-impoverished soils favored silica-based defenses. Both defense types were negatively correlated at the community and individual species level. Our results suggest a tradeoff among these two leaf defense strategies based on the strength and type of nutrient limitation, thereby opening up new perspectives for the resource availability hypothesis and plant defense research. This study also highlights the importance of silica-based defenses under low P supply.

Introduction

The resource availability hypothesis (RAH) (Coley *et al.* 1985) predicts that plant species adapted to resource-rich environments will have rapid growth rates and leaf turnover, high leaf nutrient concentrations, but low levels of anti-herbivore defenses. By contrast, the benefits of allocating resources to anti-herbivore defenses become advantageous for species adapted to nutrient-poor environments, because biomass loss by herbivory represents a significant loss of scarce nutrients (Coley *et al.* 1985; Endara & Coley 2011). This theory has been primarily explored with regard to secondary metabolites (e.g. phenolics), plant mechanical properties and nitrogen (N)-based defenses, while silica-based defenses have received far less attention (Endara & Coley 2011). For instance, in a meta-analysis testing the global consistency of the RAH (Endara & Coley 2011), the deposits of silica bodies in plants as defense mechanism (McNaughton *et al.* 1985; Hartley & DeGabriel 2016) represented only 1% of statistical tests considered in the literature (Massey *et al.* 2007). This highlights the fact that Si-based defenses have been understudied by plant ecologists, despite representing one of the earliest anti-herbivore plant adaptations (Trembath-Reichert *et al.* 2015; Deshmukh *et al.* 2020).

Vascular plants can accumulate silicon (Si) in concentrations exceeding those of the major nutrients (0.1 to 10% of dry weight; Epstein 1994). Taken up as monosilicic acid (H_4SiO_4) from the soil solution, Si is translocated to sites of rapid transpiration, where it polymerizes as amorphous hydrated silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) between cell walls and the lumen, and in extracellular and intercellular spaces of the leaf epidermis (Kumar *et al.* 2017; de Tombeur *et al.* 2020a). This mechanism of biosilicification has occurred in land plants for over 400 million years (Trembath-Reichert *et al.* 2015) and provides numerous benefits to plants, including resistance to abiotic stresses such as metal toxicity, salinity, nutrient deficiency or water stress (Cooke & Leishman 2016; Debona *et al.* 2017; Frew *et al.* 2018). Biosilicification also reduces herbivory by increasing leaf abrasiveness, which reduces penetration and chewing, and by decreasing the digestibility and palatability of leaves (Massey & Hartley 2006, 2009; Massey *et al.* 2006, 2007; Johnson *et al.* 2020). Besides physical defenses, biosilicification has been linked to anti-herbivore phytohormonal signaling through the modulation of jasmonic acid, salicylic acid and ethylene, resulting in modified emissions of volatile organic compounds (Coskun *et al.* 2019; Leroy *et al.* 2019). Although the impact of silica-based defenses on herbivory has mainly been studied in Poales (e.g., grasses), it is now well-established that the positive role of Si in biotic stresses is not restricted to Si-accumulating families (Fauteux *et al.* 2006; Deshmukh *et al.* 2013; Katz 2014; Johnson *et al.* 2019; Putra *et al.* 2020). Other taxa can have [Si] as high as in grasses (Hodson *et al.* 2005; Katz 2014), Si transporters have been identified in some legume species (Deshmukh *et al.* 2013), and it has recently been demonstrated that Si is an effective defense against herbivory in soybean (Fabaceae), even at moderate leaf [Si] (Johnson *et al.* 2019). Despite these evidences, the expression of Si-based defenses along soil fertility gradients remains underexplored, although we might expect them to increase with declining soil fertility as predicted by the RAH.

Besides silica-based defenses, one of the most important classes of plant defenses are phenolics compounds, which have been shown to co-vary with silica-based defenses (Cooke & Leishman 2012). In accordance with the RAH, high levels of phenolic compounds have long been considered as having adaptive value for plants growing on infertile soils (Bryant *et al.* 1983; Coley *et al.* 1985; Northup *et al.* 1995; Hättenschwiller *et al.* 2003; Kraus *et al.* 2004). It has

been proposed that N limitation leads to a greater expression of phenol-based defenses than phosphorus (P) limitation, because N limitation impacts the phenylpropanoid pathway more strongly, which is involved in the synthesis of both aromatic amino acids and phenol compounds (Haukioja *et al.* 1998; Jones & Hartley 1999; Wright *et al.* 2010). While this hypothesis received support in some studies (Wright *et al.* 2010; de Long *et al.* 2016), others have instead found that P limitation significantly increases plant phenol synthesis (Hättenschwiler *et al.* 2003; Sampedro *et al.* 2011; Zhang *et al.* 2012). However, these studies generally considered one single species and/or performed fertilization to simulate nutrient limitations, while community-level studies in natural environments have not been conducted. Strong natural shifts from N to P limitation of plant productivity that occur during long-term soil and ecosystem development (Vitousek *et al.* 1993; Laliberté *et al.* 2012) might therefore impact plant phenol synthesis differently in species-rich plant communities, but this has not been explored. Furthermore, the potential covariation of phenolic foliar defenses with silica-based ones (Cooke & Leishman 2012) has never been studied along soil fertility gradients.

We determined leaf Si and total phenol concentrations in plants growing along the 2 million-year Jurien Bay soil chronosequence in south-western Australia (Turner & Laliberté 2015). This long-term soil chronosequence comprises a series of coastal dunes within a global biodiversity hotspot, supporting species-rich shrubland under a Mediterranean climate (Zemunik *et al.* 2016). It exhibits an extreme gradient of soil fertility in terms of rock-derived nutrients (P, calcium, magnesium, potassium), and shifts from N to P limitation of plant productivity as soils age (Laliberté *et al.* 2012; Hayes *et al.* 2014). Leaf concentrations of major plant nutrients decrease sharply with ecosystem age (Hayes *et al.* 2014; de Tombeur *et al.* 2020c), and plants converge towards highly efficient nutrient-use strategies on the oldest, most nutrient-impoverished soils (Hayes *et al.* 2014; Guilherme Pereira *et al.* 2019). The youngest soils can also be considered infertile from an N standpoint since their N capital has not yet built up and plant growth on these soils is limited by low N availability (Laliberté *et al.* 2012; Hayes *et al.* 2014). Long-term soil chronosequences that cover both the progressive and retrogressive phases of ecosystem development provide exceptionally strong natural soil fertility and productivity gradients (Vitousek 2004; Peltzer *et al.* 2010) along which predictions of the RAH can be tested. Furthermore, the stoichiometric shifts from N to P limitation of plant growth that occur along such retrogressive soil chronosequences (Peltzer *et al.* 2010; Laliberté *et al.* 2012; Hayes *et al.* 2014) allow us to explore extensions of the RAH about the type of foliar defenses that are expressed depending on the type of nutrient limitation (e.g. N vs P on plant phenols: Wright *et al.* 2010; de Long *et al.* 2016), which have never been explored.

In accordance with the RAH, we hypothesized a greater expression of anti-herbivore defenses in plants growing on the oldest (P-limiting) and the very youngest (N-limiting) soils, compared with the intermediate-aged and most fertile soils where plant productivity, N and P availability peak (Laliberté *et al.* 2012, 2014), but further hypothesized that the type of defense most strongly expressed would depend on the type of nutrient limitation. Specifically, we hypothesized higher leaf phenol concentrations on the younger soils given the expected impact of N limitation on the phenylpropanoid pathway, whereas we expected silica-based defenses to be most strongly expressed in the oldest, P-impoverished soils. We expect the community-level patterns to be mostly driven by changes in plant species composition since the Jurien Bay chronosequence is characterized by a strong species turnover (Zemunik *et al.* 2016). This species turnover reflects

the expression of selective edaphic forces acting on a species-rich regional flora over an ecological time scale (Laliberté *et al.* 2014).

Materials and methods

Study area and site description

The 2 Ma Jurien Bay chronosequence is located in south-western Australia, approximately 200 km north of Perth (Fig. S1), and is described in detail in Laliberté *et al.* (2012) and Turner & Laliberté (2015). The chronosequence, part of the Swan Coastal Plain, comprises a series of dunes parallel to the coast, formed by periodic interglacial sea-level high-stands since the Early Pleistocene/Late Pliocene (Kendrick *et al.* 1991), with a clear gradient of soil age with increasing distance from the Indian Ocean. The dunes comprise three units: the Quindalup dunes date from the Holocene (up to 7 ka), the Spearwood dunes from the Middle Pleistocene (120 to 500 ka) and the Bassendean dunes from the Early Pleistocene or Late Pliocene (~2 Ma) (McArthur & Bettenay 1974; Playford *et al.* 1976). The parent material of the dunes is calcareous sand from the nearshore coastal environment (Turner & Laliberté 2015). The climate is Mediterranean, with a mean annual temperature of 19°C, mean annual rainfall 533 mm and potential annual evapotranspiration of 1433 mm, which results in a water balance of -900 mm yr^{-1} (data from the Jurien Bay Bureau of Meteorology from 1968 to 2015 in Turner *et al.* (2018).

We selected the same five chronosequence stages as in Hayes *et al.* (2014); these include both the early and retrogressive phases of long-term ecosystem development. The main soil properties of these five stages can be found in Table 1. Soil total P and carbonate concentrations, cation exchange capacity and pH-CaCl₂ continually decrease with increasing soil age. Soil total N concentrations increase from stage 1 to stage 2 during the progressive phase of ecosystem development, then decrease towards the last stages during the retrogressive phase (Laliberté *et al.* 2012; Turner & Laliberté 2015). Plant growth is most strongly limited by low N availability in the early stages, and by P availability in the advanced stages (Laliberté *et al.* 2012; Hayes *et al.* 2014). Previous studies showed that plant-available [Si] is low in the early stages of soil development, increases in stage 4 in the Spearwood dune system, and finally decreases in the oldest stage of soil development, where it is controlled by intense biocycling (Table 1) (de Tombeur *et al.* 2020b, c).

Site selection

For each chronosequence stage, we randomly selected five plots (10 m × 10 m each) among the 10 plots already characterized for soil and vegetation in previous studies (Hayes *et al.* 2014; Zemunik *et al.* 2016) (Fig. S1). The plots were originally selected using a random stratified sampling design (Zemunik *et al.* 2016). To characterize vegetation, seven 2 m × 2 m subplots were randomly positioned in each plot in which all individuals of all vascular plant species were counted (Zemunik *et al.* 2016). The percent canopy cover of each species was estimated, and the relative cover of each species was calculated as a fraction of the total canopy cover over the seven subplots (Zemunik *et al.* 2016).

Sampling procedure

In the 25 plots selected, we sampled leaves according to two procedures. First, we sampled leaves from one individual plant for each of the 10 most-abundant species of each plot as defined in Zemunik *et al.* (2016). The number of leaves sampled per individual was adapted according to

their mass, but was never less than 10. Occasionally, a species originally included in the 10 most-abundant species was not found on the plot, which resulted in less than 10 species for some plots (Table S1). The 234 species sampled with this first procedure still accounted for 57% to 88% of the total cover of each plot (Table S1). The community-level analyses were performed only on these species. Second, we systematically sampled the species belonging to nine families, even if they were not included in the 10 most-abundant species, in order to study family-level variation in leaf [Si] and [phenols], following the same sampling procedure: Asparagaceae, Cyperaceae, Ericaceae, Fabaceae, Haemodoraceae, Myrtaceae, Poaceae, Restionaceae and Rhamnaceae (Table S2). These families were selected because they were well represented and found at all stages of the chronosequence (Zemunik *et al.* 2016), and likely had contrasting [Si] based on known phylogenetic patterns (Hodson *et al.* 2005). In total, 298 leaf samples belonging to 24 families were collected (Tables S1 and S2).

All leaf material was collected over two weeks in November 2018. Leaves were sampled from one healthy mature individual plant per species in each plot; when an individual did not provide sufficient biomass for analysis (e.g., Poaceae spp.), leaf samples from several individuals within the plot were combined.

Leaf analyses

Leaves were washed with distilled water, dried at 70 °C for 48 h and finely ground. Leaf material (0.5 g) was placed in a porcelain crucible and calcinated at 450°C for 24 h. The weight after calcination was used to calculate the ash content. The ash was mixed with 1.6 g lithium-metaborate and 0.4 g of lithium-tetraborate in a graphite crucible and heated at 1000 °C for 5 min (Chao & Sanzalone 1992). The bead was then dissolved in 15% HNO₃ and the concentrations of Si, P, calcium (Ca), magnesium (Mg) and potassium (K) were determined by inductively coupled plasma-optical emission spectrometry (Agilent Technologies, 700 series ICP-OES). Phenolic compounds were extracted from a 0.25 g ground sample stirred with 10 mL of 70% acetone for 30 min (Salminen & Karonen 2011; Schaller *et al.* 2012; Bettaieb Rebey *et al.* 2020). Total phenols were determined in triplicate as described in Salminen & Karonen (2011) using a Folin-Ciocalteu assay with gallic acid monohydrate as standard (Merckx, Darmstadt, Germany). Total phenol concentrations were expressed as g of gallic acid equivalents (GAE) per kilogram of dry weight.

Soil sampling and analyses

In order to determine how Si availability in soils affected species-level variations in leaf [Si], three soil samples (top 20 cm) were taken in each of the 25 plots, for a total of 75 soil samples. Samples were air-dried and sieved (< 2 mm). The pool of ‘plant-available Si’ was determined by extraction in 0.01 M CaCl₂ (Haymsom & Chapman 1975; Sauer *et al.* 2006). Soil was shaken for 5 h in a 1:10 soil-to-solution ratio, filtered (cellulose filter, pore size < 2 μm, Healthcare Whatman™), acidified with 50 μL of ultrapure 65% HNO₃, and stored in darkness at 4°C prior to Si determination by ICP-OES.

Data analyses

To characterize leaf [phenols], [Si], [Ca], [Mg], [K] and [P] in plant communities across the chronosequence, we calculated the mean values of the 10 most-abundant species per plot, weighted or not by their relative canopy cover. The cover-weighted mean (CWM) was calculated as follows (Garnier *et al.* 2004; Violle *et al.* 2007):

$$CWM = \sum_{i=1}^S t_i \times RC_i$$

where t_i and RC_i are, respectively, the value of the trait t and its relative cover RC for a species i and S is the number of species.

The differences in plant-available [Si], leaf [phenols], [Si], [P], [Ca], [Mg] and [K] across the chronosequence stages were tested by one-way analysis of variance (ANOVA), followed by *post-hoc* multiple comparison (Fisher's Least Significant Difference [LSD] tests). When these analyses considered all individuals together (i.e., not the mean and CWM of the plant communities), we treated species and plots as a random factor (mixed-effect models). We tested the relationships between leaf [Si], [phenols] and major soil properties (total P, total N, ratio soil N:P) with linear mixed-effect models, treating plot and species as random factors when all individuals were considered together, and treating chronosequence stage as a random factor when the means and CWM of the 25 plots were considered. We also explored relationships between leaf [Si], [phenols] and foliar nutrient concentrations through Pearson tests of correlation. For the nine plant families selected, we tested the differences in leaf [phenols] and [Si] across the chronosequence stages using mixed-effect models with species and plot as random factors, followed by Fisher's LSD tests, and we tested the relationships between leaf [Si] and [phenols] with linear mixed-effect models (with plot and species as random factors). Additionally, we studied intraspecific variation for seven taxa, and detailed explanation of the statistical analyses used is presented in the Supplementary Information. Finally, a t-test was performed to examine the differences in leaf [Si] between dicots and monocots. All residuals were visually inspected for heteroscedasticity and appropriate transformations were performed to meet the model assumptions. All analyses were conducted in R using the 'nlme' (Pinheiro *et al.* 2020) and 'multcomp' (Hothorn *et al.* 2008) packages.

Results

Community-level leaf concentrations of Si, total phenols and major nutrients across the chronosequence stages

Leaf [Si] increased with increasing soil age, whether all individuals were considered together or the means and CWM of the 25 communities (Fig. 1a). The mean leaf [Si] within the 25 communities increased from stage 1 ($0.5 \pm 0.2 \text{ g kg}^{-1}$) to stages 2 and 3 (1.6 ± 0.4 and $2.4 \pm 0.4 \text{ g kg}^{-1}$), and then to stages 4 and 5 (3.3 ± 1.2 and $4.2 \pm 1.3 \text{ g kg}^{-1}$). The CWM followed the same pattern (Fig. 1a). The CWM leaf [Si] was negatively correlated to soil total P and positively correlated to soil N to P ratio (Fig. 2a), as for the means (Fig. S2).

By contrast, leaf [phenols] decreased with increasing chronosequence stage, whether all individuals were considered together or the means and CWM of the 25 communities (Fig. 1b). However, only stages 1-2 and 5 were significantly different from each other when all individuals were considered together. The mean leaf [phenols] decreased from stage 1 ($48.9 \pm 5.5 \text{ GAE g kg}^{-1}$) to stage 5 ($42.6 \pm 3.9 \text{ GAE g kg}^{-1}$), as did the CWM (from 51.1 ± 5.1 to $42.9 \pm 3.9 \text{ GAE g kg}^{-1}$). The CWM leaf [phenols] was positively correlated to soil total P and negatively correlated to soil N to P ratio (Fig. 2b), as for the means (Fig. S2).

Leaf [phenols] and [Si] were correlated negatively with each other when all individuals were considered (Fig. 3a), with the means and CWM of each community (Fig. 3b, c), and when

species means were considered (Fig. S3). Leaf [ash], [Ca], [Mg], [K] and [P] decreased with increasing soil age (Fig. S4). Major leaf nutrient concentrations, including P, were therefore negatively correlated with leaf [Si], whether all samples were considered individually or as means and CWM of the 25 communities (Fig. 3d).

Family and species-level leaf concentrations of Si and total phenols across the chronosequence

Within the Cyperaceae, leaf [Si] significantly increased with increasing soil age while leaf [phenols] decreased, and both defense types were negatively correlated through species replacement across the chronosequence (Fig. 4). In other plant families, leaf [Si] increased or was constant with increasing soil age, while the opposite was found for [phenols], but a significant negative relationship between both defense types was identified only for the Restionaceae (Fig. S5). For the seven species for which intra-specific variation was assessed, the mixed-effect models showed that leaf [Si] significantly increased with increasing chronosequence stage while the opposite was found for [phenols] (Table S3).

Impact of species replacement, intra-specific and inter-specific variation on the community-level leaf [Si] and [phenols]

Soil plant-available [Si] was lowest in stages 1 and 2 ($1.5 \pm 0.4 \text{ mg kg}^{-1}$), increased in stage 3 ($4.0 \pm 0.3 \text{ mg kg}^{-1}$), then in stage 4 ($9.4 \pm 1.0 \text{ mg kg}^{-1}$), before it decreased at the oldest stage ($4.3 \pm 0.2 \text{ mg kg}^{-1}$) (Table S4). Leaf [Si] was positively related to plant-available [Si] for three species of the seven (*Lepidosperma calcicola*, *Desmocladus asper* and *Acacia lasiocarpa* var. *lasiocarpa*) for which intra-specific variations were considered (Table S5). These species tended to accumulate more Si than those that did not show a correlation with plant-available [Si]. Despite this relationship between plant-available [Si] and leaf [Si] at the species-level (more pronounced for Si-accumulating species, with the exception of *Conostylis candicans* subsp. *calcicola*), the increase of the CWM leaf [Si] with increasing soil age was primarily driven by changes in the dominant plant families across the chronosequence (Fig. S6). The increase of the mean and CWM leaf [Si] with increasing soil age was not only driven by changes in the dominant families, but also within families and genera, which is described as Supplementary Results.

The plant families with the strongest contribution to the CWM leaf [phenols] strongly differed across the chronosequence (Fig. S6), highlighting the importance of species replacement on the patterns observed at the community level. In contrast to leaf [Si], the contribution of each family to the CWM [phenols] was proportional to its relative cover, highlighting less variation in [phenols] among families.

Discussion

Overall, our results do not support the main prediction of the RAH, since investments in the two anti-herbivore defenses considered were not the lowest at the most fertile soils, where both N and P availability and plant productivity peak (stages 2 and 3) (Laliberté *et al.* 2012). Instead, community-level leaf [Si] was highest on old, nutrient-impoverished soils, where P limits plant productivity, while leaf [phenols] were the highest on young soils, where plant productivity is limited by N, and both defenses were negatively correlated with each other (Fig. 5). Nevertheless, only two types of chemical defense were considered in this study and the hypothesis of an overall greater investment in defenses in the least fertile soils as predicted by the RAH should consider

the full array of anti-herbivore defenses, including structural, physical and qualitative chemical defenses (Aplin & Cannon 1971; Moles *et al.* 2013; Lambers & Oliveira 2019).

The use of soil chronosequences allows us to minimize effects of other ecosystem properties (e.g., climate, topography, parent material, salt content) beyond soil age and associated changes in major nutrients (e.g. N, P) that might influence leaf [Si] and [phenols], yet we cannot completely rule out other covarying factors. In particular, soil pH declines along the chronosequence which could indirectly impact plant-available [Si] (de Tombeur *et al.* 2020b). However, our previous work has shown that community-level changes in foliar [Si] with soil age across this chronosequence primarily arise from plant species turnover toward those that accumulate foliar [Si] as soil P availability declines, and not directly because of pH-induced changes in Si availability (de Tombeur *et al.* 2020c). Similarly, since soil nutrient availability has a greater impact on plant phenol concentrations than soil pH (Kraus *et al.* 2004), we interpret the changes in foliar [Si] and [phenols] mainly in terms of changes in soil N and P availability that are the major drivers of plant community assembly along the Jurien Bay chronosequence (Laliberté *et al.* 2014).

Convergence towards silica-based defenses during ecosystem retrogression

Our results show a convergence towards plants having higher leaf [Si] on P-impoverished soils, during ecosystem retrogression. Indeed, despite the decrease of plant-available [Si] in the last stage induced by soil desilication (de Tombeur *et al.* 2020b), the intense cycling of highly-soluble plant-derived Si in the oldest soils allows maintenance of high leaf [Si] (de Tombeur *et al.* 2020c). The convergence towards plants having higher leaf [Si] with increasing nutrient depletion was accompanied by a strong decrease in leaf macronutrient concentrations, including N (Hayes *et al.* 2014; Guilherme Pereira *et al.* 2019), and an increase in leaf mass per area (LMA) and leaf dry matter content (LDMC) along the Jurien Bay chronosequence (Guilherme Pereira *et al.* 2019). These traits highlight the convergence towards slow-growing plants adapted to P-impoverished soils and nutrient-poor environments with increasing soil age (Lambers & Poorter 1992; Wright *et al.* 2004; Garnier & Navas 2013; Reich 2014). Their higher leaf [Si] suggests that these species have evolved towards higher levels of silica deposits to minimize biomass and nutrient loss by herbivores in these nutrient-poor environments, in line with the RAH (Coley *et al.* 1985). Following this hypothesis, the likely longer leaf lifespan of these species could partly explain their higher expression of silica-based defenses, since Si accumulates as a leaf ages (Motomura *et al.* 2002).

An alternative but not necessarily mutually exclusive hypothesis could be mitigation of P stress. The first evidence for a positive effect of Si for plants growing under P stress came from the Rothamsted Experimental Station (Hall & Morison 1906). Since then, other studies have made similar observations (Ma & Takahashi 1990; Neu *et al.* 2017) and Quigley *et al.* (2020) recently proposed that high levels of biosilicification might be an adaptation to resource-poor environments. Although the mechanisms proposed are numerous and diverse, the positive role of Si under P stress is becoming clearer (Kostic *et al.* 2017), and could explain the convergence towards species having higher leaf [Si] with increasing P depletion. Overall, our results suggest that leaf Si is associated with the ‘slow’ end of the leaf economics spectrum (Wright *et al.* 2004; Reich 2014), with species having long-lived leaves with low macronutrient concentrations and high LMA and LDMC, but this hypothesis requires further investigation.

The increase of the CWM leaf [Si] from the P-limited ecosystem in stage 4 is partly explained by Proteaceae having high relative cover and relatively high [Si] for dicot species (up to 6.8 g kg⁻¹ DW; Hodson *et al.* 2005). We have no information on Si transporters (Ma *et al.* 2006) in Proteaceae, but it is possible that the exudation of carboxylates in the rhizosphere by their cluster roots (Lambers *et al.* 2008) mobilizes Si from poorly-soluble minerals, thus favoring its uptake. These cluster roots release carboxylates into the rhizosphere and release P from strongly sorbed inorganic forms (Lambers *et al.* 2008), but also mobilize micronutrients such as manganese (Mn) (Lambers *et al.* 2015; Pang *et al.* 2018). Some carboxylate-releasing roots mobilize P from rocks that are highly resistant to weathering, such as quartzite (Teodoro *et al.* 2019). The relatively high leaf [Si] in some dicot species exhibiting such specialized P-acquisition strategies might therefore be explained by their mobilization of Si through carboxylate exudation into the rhizosphere. More generally, this mechanism might explain the convergence towards leaves with higher [Si] with increasing nutrient-depletion, because the relative cover of cluster-rooted species increases markedly along the Jurien Bay chronosequence (Fig. 5) (Zemunik *et al.* 2015), and other species might benefit from the carboxylate exudation of their neighbors (Lambers *et al.* 2018). This mechanism may also explain why we observed species with some of the highest leaf [Si] found in literature (up to 43 g kg⁻¹ DW for *Mesomelaena pseudostygia* at stage 4) (Schoelynck *et al.* 2010; Carey *et al.* 2017, 2019; Schaller *et al.* 2018; Ishizawa *et al.* 2019; Nakamura *et al.* 2019) although the Jurien Bay soils are among the most desilicated worldwide, with one of the lowest Si availability for plants (de Tombeur *et al.* 2020b).

Nitrogen limitation and phenol synthesis on young soils

The CWM leaf [phenols] slightly, but significantly, decreased with increasing soil age, which we interpreted as resulting from the N-limiting conditions on the youngest soils. Indeed, although some studies suggest that P limitation increases phenol synthesis (Sampedro *et al.* 2011; Zhang *et al.* 2012) including along a long-term Hawaiian soil chronosequence (Hättenschwiler *et al.* 2003), our results are in line with others showing that N limitation has a greater effect on quantitative plant secondary metabolites (Koricheva *et al.* 1998; Wright *et al.* 2010; de Long *et al.* 2016).

The apparent stronger effect of N- compared with P limitation might be explained by their different role in plant metabolism (Jones & Hartley 1999; Wright *et al.* 2010). Under N-limiting conditions, there could be a surplus of photo-assimilates because leaf growth decreases sharply, while photosynthesis continues, albeit at a somewhat slower rate (Prescott *et al.* 2020). The ammonium-N in phenylalanine, which is a precursor of both aromatic amino acids and phenolic compounds, would then be released and re-used in other N-containing molecules (Kováčik *et al.* 2007). The rest of the phenylalanine molecule then becomes available for secondary metabolic pathways, in which it is converted into C-based secondary metabolites such as phenylpropanoid derivatives (Haukioja *et al.* 1998; Koricheva *et al.* 1998; Jones & Hartley 1999). This might explain why plants growing on the young N limited environments had higher [phenols], eliminating a surplus of photo-assimilates while conserving N. Under P-limiting conditions, however, any surplus of photo-assimilates would be exported from the chloroplasts as P-containing compounds, and converted into sucrose via reactions that release P, which is retained and reused (Stitt & Quick 1989). Therefore, P-limiting conditions might not affect the pathway of secondary metabolite synthesis in a way that N limitation does (Koricheva *et al.* 1998; Wright *et al.* 2010). Although the primary function of plant phenolic compounds may be the release of N, rather than defense against herbivores, it does make leaves less palatable for mammalian

herbivores such as kangaroos (Rafferty *et al.* 2005, 2010) on the youngest N-limited stages of the Jurien Bay chronosequence (Fig. 5).

Conclusions and Perspectives

Leaf [phenols] and [Si] were negatively correlated when considering the means and CWM of each community, all the individuals together, and within the Cyperaceae and Restionaceae family, which suggest a tradeoff between both leaf defense strategies. This pattern was observed previously (Cooke & Leishman 2012; Moles *et al.* 2013; Frew *et al.* 2016; Simpson *et al.* 2017; Waterman *et al.* 2020), but never along a soil resource gradient. Here, the species growing on older soils and adapted to nutrient-poor environments tend to favor silica accumulation over the synthesis of phenols compared with species growing on younger soils.

Raven (1983) calculated that, on a weight basis, the energetic cost of incorporating 1 g of lignin is about 27 times higher than that of incorporating 1 g of SiO₂. Given the lower metabolic costs of incorporating Si compared with other C-based compounds having similar functions (cellulose, lignin, phenols) (Schoelynck *et al.* 2010; Cooke & Leishman 2012; Klotzbücher *et al.* 2018; de Tombeur *et al.* 2020a), investing in silica as a defense mechanism (and eventually as leaf support for Cyperaceae) would make sense from an energetic standpoint on the oldest and most nutrient-depleted soils, where plants converge towards the ‘slow’ end of the leaf economics spectrum (Reich 2014; Guilherme Pereira *et al.* 2019). This energetic gain would save resources for other key aspects of the plant life cycle like growth and reproduction during ecosystem retrogression, which is key in these highly-infertile environments (Lambers 2014). Further studies should now investigate other types of anti-herbivore defenses to better evaluate the overall investment in defenses as a function of soil fertility along the Jurien Bay chronosequence. This is important given that plants display a wide range of defense traits, without clear evidence of tradeoffs between them globally (Moles *et al.* 2013). Overall, our study suggests that the type of nutrient limitation (i.e., N vs P) may induce tradeoffs in plant defense strategies, opening up new perspectives about the role of soil nutrient stoichiometry in the expression of plant defenses.

Acknowledgements

We thank the Western Australian Department of Biodiversity, Conservation and Attractions for permission to sample along the Jurien Bay chronosequences and for access to these outstanding ecosystems. This work would not have been possible without the invaluable help of Jean-Charles Bergen, Nea Fatimata and Evelyne Tanoh whom we sincerely thank. J-T.C and F.dT were supported by ‘Fonds National de la Recherche Scientifique’ of Belgium (FNRS; Research Credit Grant for the project SiCliNG CDR J.0117.18).

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Figures

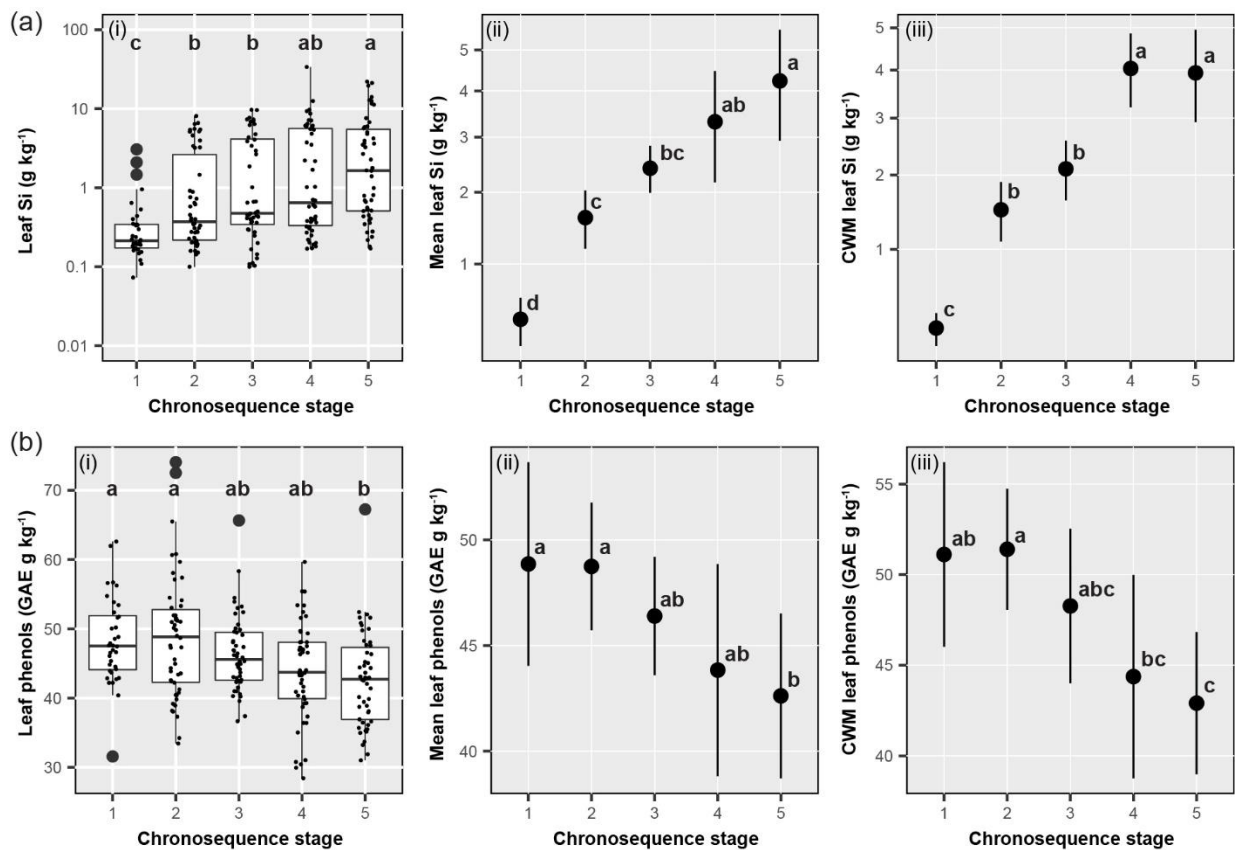


Fig. 1 – Leaf silicon (Si) concentrations across the chronosequence stages considering all individuals (i) and the means (ii) and cover-weighted means (CWM) (iii) of the five plots per stage (a). Leaf total phenol concentrations for the same chronosequence stages and plots (b). In the box-plots, small black dots represent each individual and large black dots represent outliers (outside 1.5*inter-quartile range). In the second and third panels, black dots indicate means and bars show 95% confidence intervals (n=5). In (a), Fisher LSD groupings ($p < 0.05$) were performed on log-transformed data for the first plot and on root-square-transformed data for the two others, as the scales of the axes.

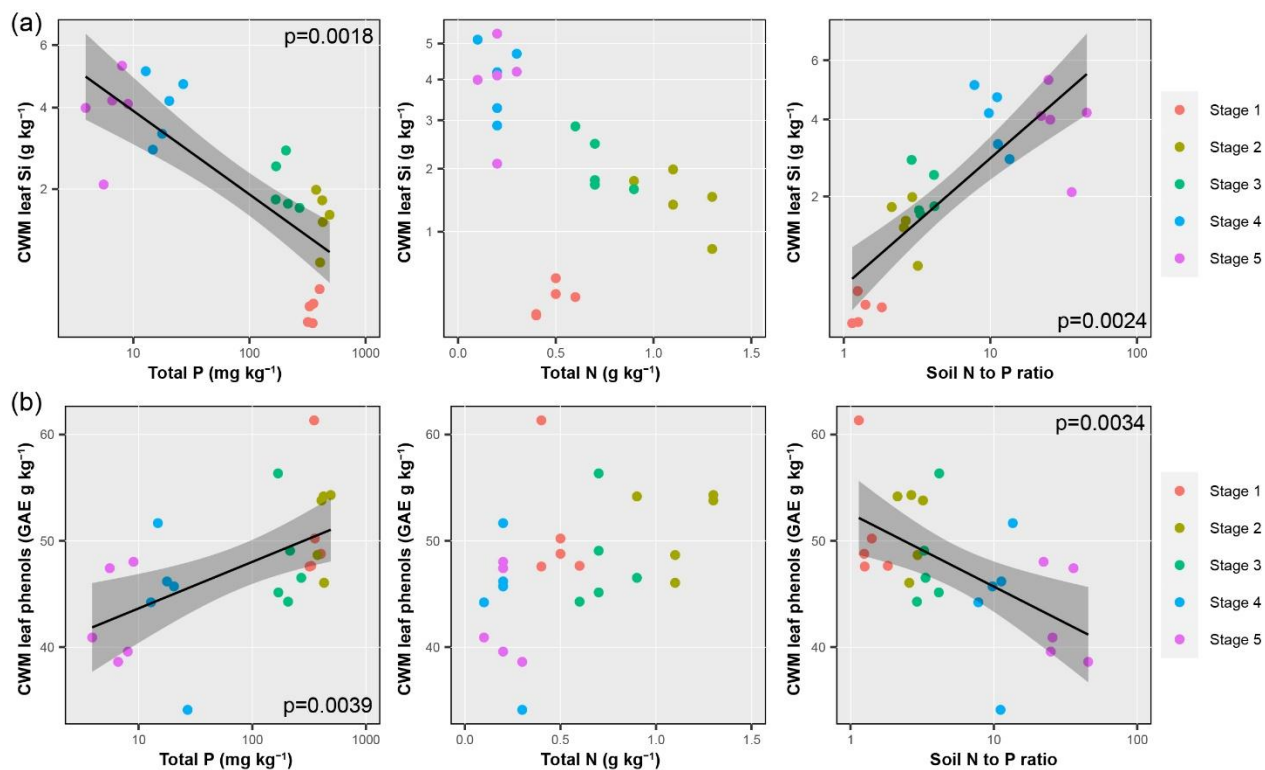


Fig. 2 – Soil total phosphorus (P) concentrations, total nitrogen (N) concentrations and soil N to P ratio versus cover-weighted mean (CWM) leaf silicon (Si) concentrations (a) and CWM leaf total phenol concentrations (b) ($n = 25$ plots). Black lines indicate the regression lines between both variables, shaded areas represent 95% confidence interval of the regression and colors of the circles indicate the chronosequence stages. Axes were log-transformed for soil total P concentration and N to P ratio and root-square-transformed for CWM leaf Si concentration. The p-values of the corresponding linear mixed-effect models are indicated if < 0.05 . Regression lines were removed if the model p-values were > 0.05 .

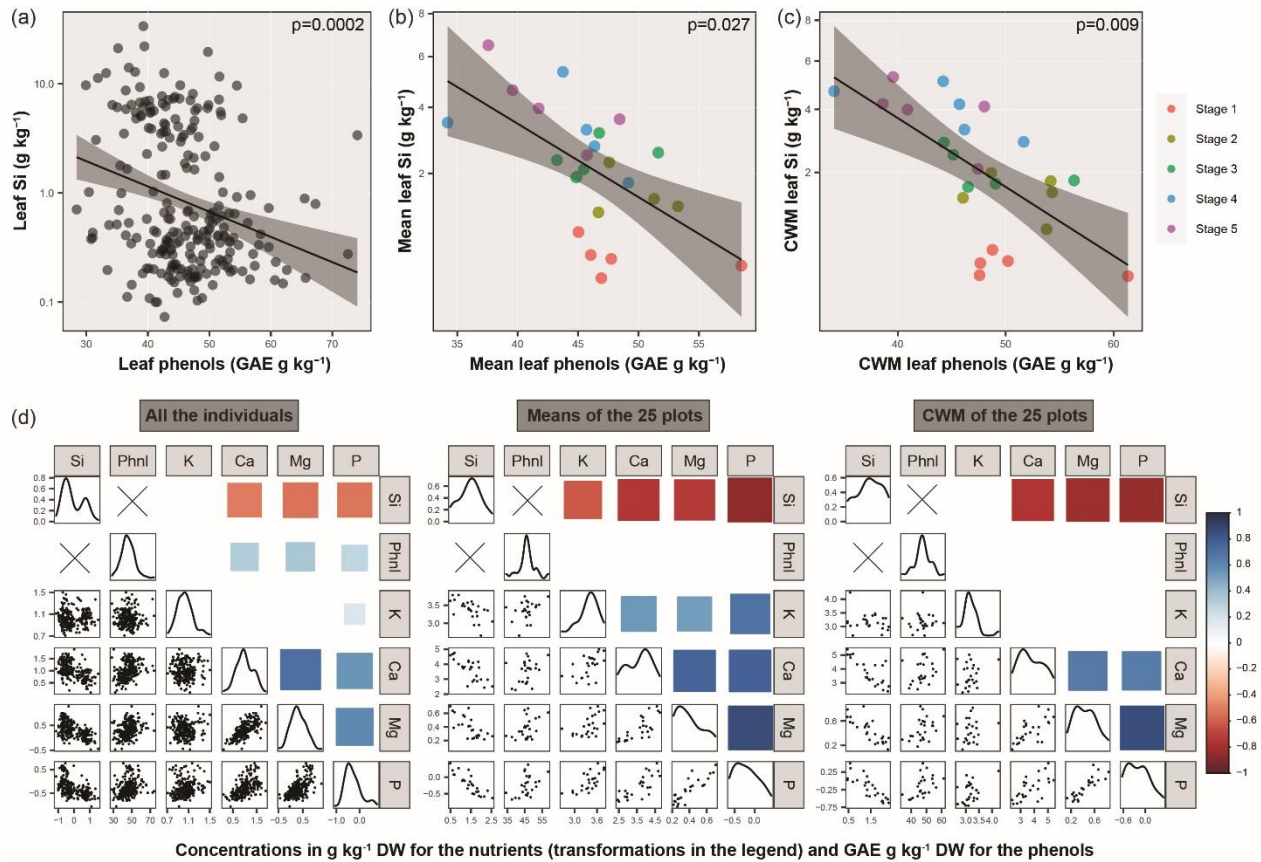


Fig. 3 – Leaf total phenol concentrations versus leaf silicon (Si) concentrations considering all individuals (a) and the means (b) and cover-weighted means (CWM) (c) of the five plots per stage. Scatterplot correlation matrix of leaf concentrations of Si, total phenols (Phnl), potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P) considering all individuals, the means, and CWM of the five plots per stage (d). In (a), (b) and (c), black lines indicate the regression lines between both variables, shaded areas represent 95% confidence interval of the regression and colors of the circles indicate the chronosequence stages. Y-axes were log-transformed in (a) and root-square-transformed in (b) and (c). The p-values of the corresponding linear mixed-effect models are indicated if < 0.05 . In (d), the size and color of the squares represent Pearson's correlation coefficient. All correlations having a p-value > 0.01 are represented by a blank space. Plots on the diagonal represent the distributions of each variable. Concentrations are in gram per kg dry weight for the nutrients and gallic acid equivalent (GAE) gram per kg dry weight for total phenols. In (d), axes were log-transformed for all variables, except for leaf [phenols] in the first plot, while root-square-transformed for leaf [Si], [Ca] and [K] and log-transformed for leaf [P] and [Mg] in the second and third plots.

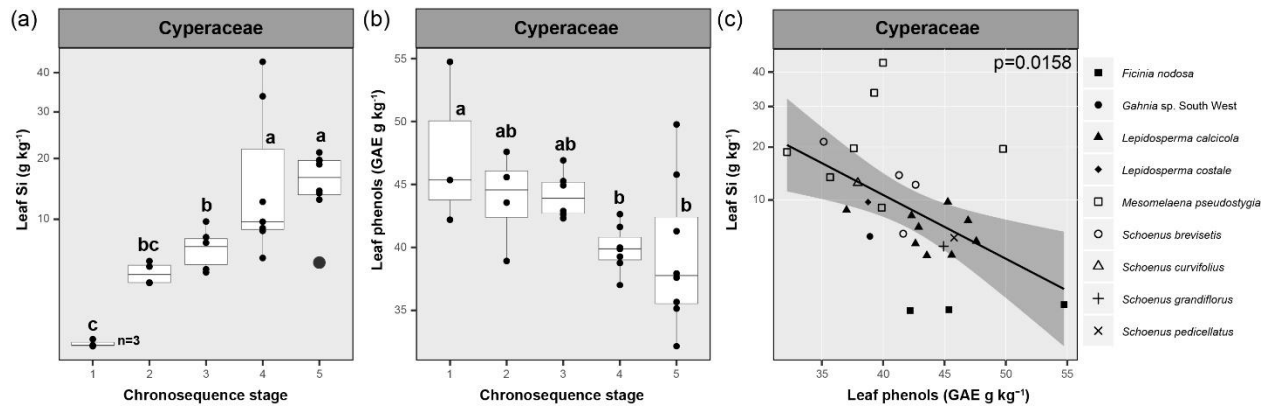


Fig. 4 – Leaf silicon (Si) (a) and total phenol concentrations (b) across the chronosequence stages for Cyperaceae, and relationship between both for the same individuals (c). In (a) and (b), the small black dots represent each species and large black dots represent outliers (outside 1.5*inter-quartile range). In (a), Fisher LSD groupings ($p < 0.05$) were performed on root-square-transformed data, as the scale of the axes. In (c), the y-axis was root-square-transformed; each symbol represents a different species, the black line indicates the regression line between both variables, the shaded area represents 95% confidence interval of the regression, and the p-value of the corresponding linear mixed-effect model is indicated.

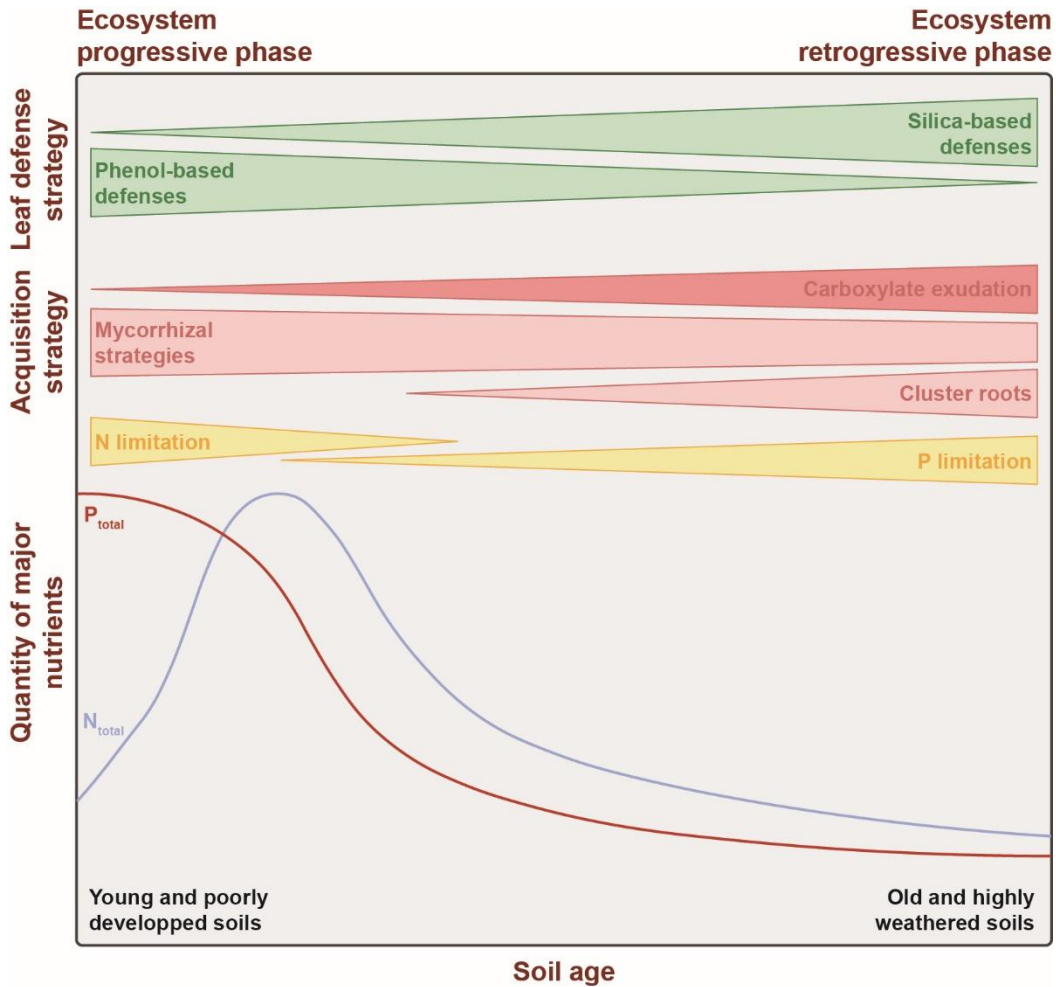


Fig. 5 – Schematic representation (adapted from Lambers *et al.* 2008) of changes in soil total phosphorus (P) and nitrogen (N), P-acquisition strategies, and leaf defense strategies during long-term ecosystem development. Changes in soil P and N are based on Hayes *et al.* (2014). At the Jurien Bay chronosequence, soil total P concentration continually declines with increasing soil age, from about 430 to 6 mg kg⁻¹, while total N concentration increases from 0.5 to 1.2 g kg⁻¹ in the young soils where total [P] is relatively high, then declines to 0.2 g kg⁻¹ in the oldest soil, resulting in a shift from N to P limitation of plant growth. The changes in nutrient-acquisition strategies were originally suggested by Lambers *et al.* (2008), but subsequently confirmed in Zemunik *et al.* (2015). Along the Jurien Bay chronosequence, the relative cover declines for arbuscular mycorrhizal and ectomycorrhizal species, but increases for carboxylate-releasing cluster-rooted species and others with functionally equivalent strategies. The changes in silica and phenol-based defenses are based on the present study.

Tables

Table 1 – Main properties of the five chronosequence stages used in this study. The chronosequence stages, dune system, geological formation and estimated soil age are based on Laliberté *et al.* (2012) and Turner & Laliberté (2015). The carbonate, total phosphorus (P) and nitrogen (N) concentrations, soil total N to total P ratio, pH-CaCl₂ and cation exchange capacity (CEC) are based on Hayes *et al.* (2014) and Zemunik *et al.* (2016). They result from seven soil samples (0-20 cm deep) taken in each of the five plots by chronosequence stage used in this study (n = 25 plots). The limiting nutrients are based on Laliberté *et al.* (2012) and Hayes *et al.*

Stage	Dune system	Geological formation	Estimated soil age (ka)	Carbonates* (%)	pH-CaCl ₂ [†]	CEC [†] (cmolc kg ⁻¹)	Total P [†] (mg kg ⁻¹)	Total N [†] (g kg ⁻¹)	Soil total N to total P ratio [†]	Limiting nutrients	Expected Si availability
1	Quindalup young	Safety Bay Sand	0.1 (Holocene)	75.2(3.5)	8.2(0.1)	30.5(4.5)	351.0(6.6)	0.5(0.0)	1.4(0.1)	N	Very low
2	Quindalup medium	Safety Bay Sand	1 (Holocene)	75.5(2.8)	7.8(0.0)	12.2(1.1)	424.4(8.3)	1.2(0.0)	2.7(0.4)	N, P and/or other nutrients	Very low
3	Quindalup old	Safety Bay Sand	6.7 (Holocene)	25.6(3.7)	7.8(0.0)	10.9(0.5)	205.7(7.0)	0.7(0.0)	3.6(0.5)	N, P and/or other nutrients	Low
4	Spearwood	Tamala Limestone	125 (Middle Pleistocene)	BDL	5.8(0.1)	3.6(0.2)	18.5(1.0)	0.2(0.0)	10.7(1.9)	P	Medium
5	Bassendean	Bassendean sand	>2000 (Early Pleistocene or Late Pliocene)	BDL	4.8(0.1)	2.5(0.2)	6.6(0.5)	0.2(0.0)	30.8(8.5)	P	Low

(2014). The expected silicon (Si) availability are from de Tombeur *et al.* (2020b, c).

*SE is indicated in brackets. The seven soil samples were bulked before analysis (n=5).

[†]SE is indicated in brackets (n=35).

BDL, below detections limits