Morphological and physiological adjustment to N and P fertilization in nutrient-limited Metrosideros polymorpha canopy trees in Hawaii

S. CORDELL,1,2 G. GOLDSTEIN,1 F. C. MEINZER3,4 and P. M. VITOUSEK5

1 Department of Botany, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822, USA
2 Present address: USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA
3 Present address: USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA
4 Present address: USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA
5 Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA

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Summary  Leaf-level studies of Metrosideros polymorpha Gaud. (Myrtaceae) canopy trees at both ends of a substrate age gradient in the Hawaiian Islands pointed to differential patterns of adjustment to both nutrient limitation and removal of this limitation by long-term (8–14 years) nitrogen (N), phosphorus (P) and N + P fertilizations. The two study sites were located at the same elevation, had similar annual precipitation, and supported forests dominated by M. polymorpha, but differed in the age of the underlying volcanic substrate, and in soil nutrient availability, with relatively low N at the young site (300 years, Thurston, Hawaii) and relatively low P at the oldest site (4,100,000 years, Kokee, Kauai). Within each site, responses to N and P fertilization were similar, regardless of the difference in soil N and P availability between sites. At the young substrate site, nutrient addition led to a larger mean leaf size (about 7.4 versus 4.8 cm²), resulting in a larger canopy leaf surface area. Differences in foliar N and P content, chlorophyll concentrations and carboxylation capacity between the fertilized and control plots were small. At the old substrate site, nutrient addition led to an increase in photosynthetic rate per unit leaf surface area from 4.5 to 7.6 µmol m⁻² s⁻¹, without a concomitant change in leaf size. At this site, leaves had substantially greater nutrient concentrations, chlorophyll content and carboxylation capacity in the fertilized plots than in the control plots. These contrasting acclimation responses to fertilization at the young and old sites led to significant increases in total carbon gain of M. polymorpha canopy trees at both sites. At the young substrate site, acclimation to fertilization was morphological, resulting in larger leaves, whereas at the old substrate site, physiological acclimation resulted in higher leaf carboxylation capacity and chlorophyll content.

Keywords: chlorophyll content, leaf level traits, nutrient limitation, photosynthesis.

Introduction

Limitation of plant productivity by nitrogen (N) or phosphorus (P), or both, is widespread in tropical regions. Phosphorus limitation tends to occur on old soils, whereas N limitation frequently occurs on young soils and on chronically disturbed sites (Vitousek and Howarth 1991). Traits characterizing plants native to nutrient-limited environments include slow growth, low photosynthetic capacity, low rates of nutrient uptake, and reduced leaf size (Parsons 1968, Grime 1977, Chapin 1980). Increased nutrient availability in nutrient-limited systems generally increases aboveground productivity. However, it is unclear whether this response is a result of increased leaf photosynthetic capacity or a shift in biomass allocation, thereby increasing the number of photosynthetic units. Moreover, it remains unclear how nutrient uptake and utilization are regulated in fertilized plots, or whether genetic constraints govern maximum nutrient utilization.

Relief of nutrient limitation may result in increased net CO₂ assimilation. Several studies have reported correlations between soil N availability, photosynthetic capacity and total leaf N content. For example, Field and Mooney (1986) found a tight linear relationship between mass-based N and mass-based rates of net photosynthesis over a wide range of C₃ plants, suggesting that photosynthetic capacity is strongly regulated by leaf N and is not greatly influenced by growth form or species. Although foliar N concentration and its relationship with carbon gain and leaf traits have been well studied, little is known about the relationships between morphological, biochemical and physiological responses to P-limitation in a natural environment.

To compare the consequences and implications of nutrient limitation by N and P at the leaf level, we conducted a study at both ends of a long, substrate age gradient in the Hawaiian Islands. The sites in the chronosequence form a natural fertility gradient from young, primarily N-deficient soils to older, mostly P-deficient soils, but have similar climate, and species composition (Chadwick et al. 1999). Furthermore, long-term forest fertilization treatments (8–14 years) are ongoing at both the young and old substrate ends of the chronosequence. Previous studies indicated that applications of N caused increased
forest growth at the young substrate site (Vitousek et al. 1993), and additions of P stimulated production at the oldest site (Herbert and Fownes 1995). Our objectives were to characterize the leaf-level responses of _Metrosideros polymorpha_ Gaud. (Myrtaceae), the dominant Hawaiian forest canopy species, to N and P limitation, and the effect of removal of this limitation on photosynthesis, leaf morphology and foliar nutrients and chlorophyll content. In addition, we evaluated stand-level consequences of changes in leaf characteristics.

### Materials and methods

#### Study site

This study was conducted at both ends of a well-defined and constrained substrate age sequence in the Hawaiian Islands. The two extremes of the chronosequence selected for this study were 300 and 4,100,000 years old (Table 1). Hawaii is an ideal location for studying processes at leaf, stand and ecosystem levels in the field because many of the environmental factors that regulate plant function can be kept constant, and others allowed to vary in well-defined ways (Britten 1962, Vitousek et al. 1992). Most of the parent materials of the soil have similar characteristics. The chemistry of the volcanic material that makes up Hawaii reflects that of a stationary convective plume or “hotspot” (Moore and Clague 1992), and is relatively constant on both short and long time scales (Wright and Helz 1987). The soils at the study sites have evolved from basaltic rock mixed with tephra and pumice substrate with similar initial chemistry; however, they differ in the age of the underlying substrate and in soil properties related to age. Soils at the young substrate site are little weathered and contain low amounts of N and P (Chadwick et al. 1999). Soils at the oldest site have lost most of their active minerals to weathering and leaching. These soils have little capacity to supply nutrients, particularly P, as well as little ability to retain recycled or added nutrients (Chadwick et al. 1999).

Plant community composition in Hawaii can be held constant to a striking extent. Hawaii is the most isolated archipelago on Earth, and the few natural colonists that have become established have radiated to occupy a broad range of environments (Carlquist 1980). The myrtaceous tree _Metrosideros polymorpha_, the dominant wet forest species, grows from sea level to 2500 m, from among the first woody colonists on young lava flows to the oldest substrates in the islands, and from 400 mm annual rainfall to one of the wettest places on Earth (Dawson and Stemmermann 1990). _Metrosideros polymorpha_ exists in several forms including the pubescent varieties _incana_ and _polymorpha_ and the glabrous variety _glaberrima_. The pubescent varieties make up ~95% of the _M. polymorpha_ at the young substrate site with _M. polymorpha_ var. _glaberrima_ making up the balance. _Metrosideros polymorpha_ var. _glaberrima_ is the sole variety of _Metrosideros_ at the old substrate site (Dawson and Stemmermann 1990). Even though this species has radiated to occupy environments with different physical and climatic conditions, multiple species have not formed, probably because of the efficient long distance transport of wind-dispersed seeds and the small size of the islands. The forests at both study sites have similar species composition, with _M. polymorpha_ making up more than 80% of the canopy trees.

Complete factorial fertilizations have been carried out in the 300 and 4.1 million-year-old substrate sites. The main treatments were control (no added fertilizer), N (100 kg ha$^{-1}$ year$^{-1}$, half as urea and half as ammonium nitrate), P (100 kg ha$^{-1}$ year$^{-1}$, triple superphosphate), and N plus P (100 kg N ha$^{-1}$ year$^{-1}$ + 100 kg P ha$^{-1}$ year$^{-1}$). The field design consists of four blocks of four 15 × 15 m plots at both the young (Thurston, Hawaii) and old (Kokee State Park, Kauai) substrate sites (Table 1). We conducted intensive measurements in one plot of each of four treatments at both the young and old extremes of the gradient (Control, N, P and N + P). Treatment plots were located in a homogeneous forest stand with similar soil characteristics and slopes. Leaves from the upper canopy were accessed from 10–20-m scaffolding towers (1.6 × 3.2 m) placed in the center of one plot per treatment. At least four canopy trees per plot were accessible from each tower. To ensure adequate replication, sun leaves were collected with a shotgun from three additional plots per treatment that were not accessible by towers. Morphological information was obtained from these leaves (area, mass and leaf mass per unit area (LMA)), and mean values of all four plots per treatment were used for calculations of whole-leaf physiological and biochemical measurements. Photosynthetic gas exchange measurements were obtained only from plots with towers.

#### Photosynthetic gas exchange and chlorophyll content

Net CO$_2$ assimilation (A) was determined with a portable photosynthesis system (LI-6200, Li-Cor, Inc., Lincoln, NE). A portable light source was used to maintain a constant irradiance (1600 µmol m$^{-2}$ s$^{-1}$) during measurements (Qbeam...
temperature was held constant at 20–22 °C, photon flux den-

sions, samples were ground in a ball mill, then di-

corrections for the solvent. Chlorophyll content was calcu-

were determined on three

and the decline in

with a plant canopy analyzer (LAI-2000, Li-Cor, Inc.). Three

Vertical leaf area index (LAI) was estimated nondestructively

ADJUSTMENT TO FERTILIZATION IN NUTRIENT-LIMITED TREES 45

Leaf nutrients and morphology

Five fully expanded sun leaves were collected from four trees

Leaf area index and carbon gain

Vertical leaf area index (LAI) was estimated nondestructively

Results

Leaf morphological traits

Applied separately, N and P significantly increased leaf size

Addition of N or P alone did not significantly increase foliar N

Statistical analysis

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Kitayama et al. 1997). To estimate both maximum rates of A,

The light-saturation point of *M. polymorpha* is less than 600 µmol m⁻² s⁻¹

3.5 m from ground), mid-canopy (6.5 m from ground), and

This result was especially striking at the old substrate site

Addition of N or P alone did not significantly increase foliar N concentrations on a mass or leaf area basis at the young substrate site. However, there was a significant increase in N concentration of leaves in the N, P and N + P fertilized plots at the old substrate site (Table 2). Addition of P substantially increased P concentrations (% of leaves at both sites (Table 2). This result was especially striking at the old substrate site where addition of P increased foliar P concentration from 0.05 to 0.18%. Foliar N content (mg leaf⁻¹) significantly increased in the N-fertilized plots at the young substrate site. Although N fertilization had no significant effect on foliar N content at the old substrate site, P additions significantly increased foliar P content at this site. Addition of N + P at both the young and old substrate sites increased foliar concentrations of N (relative to the addition of N or P alone).
Photosynthetic gas exchange

Effects of fertilization on net CO₂ assimilation (A) of *M. polymorpha* leaves differed between the young and old substrate sites, especially when compared on an area versus whole-leaf basis (Figures 2a and 2b). Net CO₂ assimilation of *M. polymorpha* leaves from the control plot was substantially higher at the young substrate site than at the old substrate site (Figure 2). After fertilization at the old substrate site, values of A were similar to those at the young substrate site (Figure 2a). After fertilization at the young substrate site, A remained essentially constant on a leaf area basis, but increased significantly (*P < 0.001*) when calculated on a whole-leaf basis.

The initial slope of the A–pᵢ curves measured on leaves from the young substrate site did not differ substantially across treatments (Table 3). At the old substrate site, the initial slope of the A–pᵢ curve (a non-invasive measurement of carboxylation capacity) was significantly greater in all of the fertilization treatments than in the control. For all treatments, the initial slopes of A–pᵢ curves were steeper for trees at the young substrate site than at the old substrate site. The CO₂ compensation point was essentially constant in all treatments at the young substrate site. Fertilization with N and P significantly increased the CO₂ compensation point at the old substrate site.

Net CO₂ assimilation increased with foliar N concentration at the old substrate site when both were expressed on a mass basis (Figure 3a). At the young substrate site, mass-based A was independent of foliar N concentration. Net CO₂ assimilation expressed on a whole-leaf basis increased asymptotically with total N per leaf when data for both sites were combined (Figure 3b). However, the range of variation in A associated with N differed between sites, with lower rates of net CO₂ assimilation measured at the old substrate site than at the young substrate site (Figure 3b).

A significant linear increase in mass-based A with increasing foliar chlorophyll (chl) concentration was observed at the old substrate site but not at the young substrate site (Figure 4).
ure 4a). On a whole-leaf basis, A increased with increasing chl content up to 6 µg chl leaf –1 and then remained constant at higher chl contents (Figure 4b).

Table 3. Parameters derived from the initial linear portion of $A$–$p_i$ curves for leaves of *Metrosideros polymorpha* trees growing in control and fertilized plots at the young and old substrate sites. Values are means of three curves from three trees per plot ($n = 9$). Significant effects of fertilization are indicated as: no asterisk = < 0.1, * = < 0.05, ** = < 0.01, and *** = < 0.001.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO₂ compensation point (ppm)</th>
<th>Slope of $A$–$p_i$ curve</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young substrate site (300 years old)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>65.1</td>
<td>0.054</td>
<td>0.93</td>
</tr>
<tr>
<td>N</td>
<td>67.3</td>
<td>0.049</td>
<td>0.99</td>
</tr>
<tr>
<td>P</td>
<td>58.0</td>
<td>0.057</td>
<td>0.99</td>
</tr>
<tr>
<td>N + P</td>
<td>56.8</td>
<td>0.057</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Old substrate site (4.1 million years old)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>55.2</td>
<td>0.023</td>
<td>0.99</td>
</tr>
<tr>
<td>N</td>
<td>87.1**</td>
<td>0.043***</td>
<td>0.98</td>
</tr>
<tr>
<td>P</td>
<td>72.6**</td>
<td>0.043***</td>
<td>0.99</td>
</tr>
<tr>
<td>N + P</td>
<td>63.4</td>
<td>0.036***</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Figure 2. Maximum net CO₂ assimilation rates of *Metrosideros polymorpha* sun leaves in the four treatment plots: Control (C), N-fertilized (N), P-fertilized (P) and N + P fertilized (NP) at the young and old substrate sites. Values in (a) are based on leaf surface area, and values in (b) are based on whole leaves. Bars represent means of 20 measurements per treatment ± SE. At the young substrate site, fertilization had no significant effect on area-based assimilation, whereas all fertilization treatments had a significant effect on area-based assimilation ($P < 0.001$) at the old substrate site. All of the fertilization treatments significantly affected whole-leaf measurements of net CO₂ assimilation at the young substrate site ($P < 0.001$), and the old substrate site (N, $P < 0.05$), (P, N + P, $P < 0.001$).

Figure 3. Relationships between net CO₂ assimilation and foliar N concentration and content of *Metrosideros polymorpha* based on measurements from our treatment plots at two sites. Each value represents a mean from five measurements taken from four trees per treatment plot ± SE ($n = 4$ per treatment). (a) Assimilation and foliar N measured on a mass basis; and (b) assimilation and foliar N expressed on a whole-leaf basis. A regression line was not fitted to the data from the young substrate site because the relationship was not significant at $P < 0.05$, at the old substrate site $P < 0.05$.

**LAI and carbon gain**

Fertilization increased LAI at the young substrate site (Table 1). At the old substrate site, only the N + P fertilization treatment increased LAI. Different relationships between total N per leaf and foliar N concentration were observed at the young and old substrate sites (Figure 5a). At the old substrate site, foliar N concentration ranged from 0.8 to 1.4%, but total N per leaf remained constant. In contrast, total N per leaf at the young substrate site varied substantially, whereas foliar N concentration exhibited a relatively small range of variation. Total canopy carbon gain (mean annual foliar carbon gain × LAI) increased with increasing total N per leaf at both the young and old substrate sites, but there was little overlap in canopy carbon gain between the two sites (Figure 5b). At the young substrate site, total carbon gain increased from 19.6 mol m⁻² year⁻¹ in the control plot to 55.5 mol m⁻² year⁻¹ in the
N-fertilized plot. At the old substrate site, total carbon gain increased from 13.7 mol m\(^{-2}\) year\(^{-1}\) (control) to 36.7 mol m\(^{-2}\) year\(^{-1}\) (N + P addition).

**Discussion**

Nutritional deficiency has usually been determined empirically by evaluating plant responses in terms of growth, reproductive effort or total carbon uptake to additions of the nutrient assumed to be limiting (Kimmins 1997). It has generally been accepted that, although increased availability of nutrients to a nutrient-deficient plant initially increases its growth rate, foliar nutrient concentration may exhibit little change because of a dilution effect caused by increased carbon accumulation (Ulrich and Hill 1967). When a condition of adequate nutrition is reached, or if some other nutrient or environmental factor becomes limiting to growth, uptake may continue and foliar nutrient concentration may start to increase. This picture has emerged from nutritional studies of crops and tree plantations, which usually require high amounts of nutrients to attain maximal growth rates. Native plants, and in particular native non-pioneer trees, are usually tolerant to nutrient deficiencies and tend to have relatively low growth rates. Therefore, responses of native plants to additions of limiting nutrients may differ from those of cultivated plants (Chapin 1987). For example, although *M. polymorpha* responded positively to additions of both N and P separately, the responses were not additive when N and P were applied together.

We found striking differences in the responses of *M. polymorpha* trees to nutrient additions at the young substrate site compared with the old substrate site. Increased nutrient availability at the young substrate site led to large increases in leaf size and weight, but negligible or very small increases in foliar N concentration. Although fertilization with N and P at the young substrate site has continued for more than 10 years, foliar N concentrations have never approached those observed in unfertilized plots at the 20,000-year-old Laupahoehoe site in Hawaii, where natural soil fertility is relatively high (Vitousek 1998). In contrast, N and P additions at the old substrate site did not affect leaf size and mass, but caused significant increases in foliar N and P concentrations. Because of potential variation in nutrient and carbon allocation, the concentration of a particular nutrient in a particular tissue (e.g., leaves) cannot always be used as a direct indicator of plant nu-
trient status. This was particularly evident in *M. polymorpha* at both study sites. Fertilization had no effect on leaf nutrient concentrations at the young substrate site, and at any leaf N concentration, total N content per leaf was one to two times higher at the young substrate site than at the old substrate site, as a result of increased leaf size. This finding suggests that fertilization altered the patterns of N and C allocation in leaves. For example, fertilization resulted in higher leaf C:N ratios at the young substrate site than at the old substrate site.

Mass-based net CO₂ assimilation did not increase at the young substrate site. Only whole-leaf CO₂ assimilation increased in response to nutrient addition as a result of increased leaf size. In contrast, increased nutrient availability at the old substrate site led to the expected pattern of increasing photosynthetic rates with increasing foliar N concentration (Field and Mooney 1986, Reich et al. 1997). Consistent with the increases in nutrient concentration and photosynthetic rate, chlorophyll content and carboxylation capacity tended to increase with increasing N at the old substrate site. Increased N availability at the young site, on the other hand, resulted in negligible increases in chlorophyll concentration. Because the synthesis of chlorophyll is also inhibited by nitrogen deficiency, it is possible that despite long-term fertilization, chlorophyll synthesis is still limited by nitrogen availability at the young substrate site, or it has reached a maximum value according to the genetic constraints of the leaf.

Although the response of net CO₂ assimilation to fertilization differed between the two study sites, net CO₂ assimilation per leaf exhibited the same functional relationship with N content per leaf at both sites. That is, there was a rapid increase in total carbon gain per leaf at low foliar N and then a more gradual increase at higher foliar N. However, the operational ranges of leaf carbon gain differed in trees growing at the young and old substrate sites. The *M. polymorpha* leaves at the old substrate site had lower foliar N content and tended to have lower total carbon gain compared with leaves at the young substrate site. Consequently, we believe that it is appropriate to use total N per leaf in *M. polymorpha* as an index of total potential carbon gain because it increased at both sites in response to fertilization. The increase was brought about by an increase in leaf size at the young substrate site and by an increase in mass-based N with no change in leaf size at the old substrate site. These results exemplify the need to integrate instantaneous measurements of photosynthesis with leaf morphological characteristics to understand the effect of nutrient availability on total carbon gain. For example, Küppers (1994) demonstrated that, in two species of *Eucalyptus* having different growth and photosynthetic rates, the species with the lower photosynthetic rate had a higher growth rate apparently attributable to a higher LAI and lower LMA.

Fertilization increased LAI of trees at the young substrate site, leading to increased light interception and CO₂ acquisition and hence to an increase in total aboveground biomass. Our results are also consistent with stand-level measurements showing an increase in aboveground net primary productivity (calculated from stem diameter increments and litterfall production) associated with fertilization (R. Harrington, University of Massachusetts; unpublished data). The mechanisms promoting an increase in leaf surface area without increasing carboxylation capacity in response to fertilization at the N-limited site are unknown. Because *M. polymorpha* trees do not form a dense closed canopy like continental tropical forests, competition for light interception does not appear to be an important selective pressure, potentially allowing plasticity in leaf size.

Several factors may account for the different responses of *M. polymorpha* to fertilization at the two sites. Among these are genetic differences in the two *M. polymorpha* populations, and differences in soil properties such as compaction and water-holding capacity at the two sites. With respect to genetic differences, results from previous study of morphological and physiological differences in *M. polymorpha* across an altitude gradient suggest that patterns of foliar carbon allocation in response to long-term P limitation may have a strong genetic component (Cordell et al. 1998). With respect to soil properties, foliar traits consistent with water conservation, such as small leaves, shorter leaf life span and less negative δ¹³C values have been observed at the old substrate site (data not shown), suggesting a dual water and nutrient limitation.

In conclusion, long-term fertilization enhanced productivity of *M. polymorpha* at both study sites, indicating that both sites have limited nutrient availability. Nevertheless, the mechanisms of response to addition of N and P differed between sites. At the young substrate site, increased nutrient availability resulted in large leaves with N and chlorophyll concentrations similar to those of unfertilized plants. Consequently, carboxylation capacity remained constant. Increased nutrient availability at the old substrate site, on the other hand, led to increases in photosynthetic rate per unit leaf surface area, carboxylation capacity and N and chlorophyll concentration, with no concomitant changes in leaf size. Thus, at the young substrate site, acclimation to fertilization was morphological, resulting in larger leaves, whereas at the old substrate site, physiological acclimation resulted in higher leaf carboxylation capacity and chlorophyll content.

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