Responses to CO₂ Enrichment by Two Genotypes of Arabidopsis thaliana Differing in their Sensitivity to Nutrient Availability

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The responses of two genotypes of Arabidopsis thaliana, which differ in their sensitivities to nutrients to present and predicted future CO₂ concentration were determined under rich vs. poor nutrient regimes on the basis of both single traits and the whole plant. Based on individual traits, the two genotypes responded similarly to CO₂ enrichment for all the traits measured except for rate of increase in crown diameter, for which a decrease was observed in the less nutrient-sensitive genotype grown at increased CO₂. Based on the overall response of the whole plant, by analysing groups of plant traits using multivariate analysis, the two genotypes differed substantially from one another and both responded more strongly to nutrient availability than to CO₂ concentration, especially for traits measured at harvest that related to reproductive fitness. The less nutrient-sensitive genotype also showed a weaker overall response to CO₂, and the pattern of the overall response was strikingly similar at different nutrient supply. In contrast, the more nutrient-sensitive genotype responded more strongly to CO₂ than the less nutrient-sensitive genotype, and responded differently to CO₂ at low vs. high nutrient availability.

Key words: Plasticity, CO₂ enrichment, nutrient status, nutrient × CO₂ interaction, Arabidopsis thaliana, canonical analysis.

INTRODUCTION

The expectation that atmospheric CO₂ concentration will double from pre-industrial values by the mid- to late-21st century (Conway et al., 1988; Ramanathan, 1988) has evoked much concern for the impact of elevated CO₂ on plants and ecosystems (Coleman and Morse, 1990; Ackerly et al., 1992; Bazzaz and McConnaughay, 1992; Coleman and Bazzaz, 1992). Many studies have shown a wide range of responses to CO₂ concentration in both crops and wild plants (Warrick and Gifford, 1986; Bazzaz, 1990) depending not only on CO₂ concentration but also on other abiotic and biotic factors, such as soil nutrients, temperature and moisture, and the genetics of the plants (Wray and Strain, 1986; Ackerly et al., 1992; Baker, Allen and Boote, 1992; Sivola and Ahlholm, 1992). Predicting the effects of increasing atmospheric CO₂ on plant species requires an understanding of the interaction between CO₂ and other limiting environmental factors. Because atmospheric CO₂ often limits plant photosynthetic capacity, many plants show increased photosynthetic rate and biomass in response to elevated CO₂ (Pearcy and Bjorkman, 1983; Cure, 1985). When other environmental factors, such as soil nutrients, become limiting, the photosynthetic capacity and growth of plants may be enhanced by elevated CO₂ to the same degree as that without limitation (Wong and Osmond, 1991; Gifford, 1992), or remain virtually unchanged across CO₂ concentrations (Larigaude, Hilbert and Oechel, 1988; Radoglou and Jarvis, 1992; Sivola and Ahlholm, 1992).

Differences in the ability of plants to acquire both carbon and nutrients by altering the partitioning of biomass between plant roots and shoots are thought to be responsible for the diverging results (Idso, Kimball and Mauney, 1988; Eamus and Jarvis, 1989). These inter-specific differences in response to CO₂ enrichment are expected to lead to changes in the composition of communities as a result of divergent selection on different species, and the patterns and intensities of such changes can be expected to vary with other environmental conditions (Williams, Garbutt and Bazzaz, 1988; Bazzaz et al., 1990). Much less attention has been given to the interaction between CO₂ and other factors at the intraspecific level (Wang, Lechowicz and Potvin, 1994, 1995). As the edaphic environment varies at small scales relevant to individual plants within a natural population (Lechowicz and Bell, 1991), and genotypes of a given species may differ in the amount and/or pattern of morphological and/or physiological response to environmental variations (Schlichting, 1986), it is worthwhile to examine the variation between genotypes in response to the combined effect of CO₂ and other environmental factors.

Conventionally, plant morphological and physiological traits are studied individually to assess the response of an organism to CO₂ enrichment and, not surprisingly, some traits increase with increasing CO₂ and others decrease or remain unchanged (Bazzaz, 1990, and references therein). It is fair to assume that these disparate changes in individual traits may reflect a co-ordinated overall plastic response, which represents the adjustment in phenotypic expression of a genotype under different environments, of the whole plant. Both the amount and the pattern of such a plastic response require evaluation (Schlichting, 1986). It is the

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amount and pattern of changes in these coordinated interrelationships among traits that organize and determine the performance of the organism. Measuring the amount and pattern of phenotypic plasticity among genotypes grown under different environments requires a reasonable way to synthesize the many small changes that characterize the overall response (Zhang and Lechowicz, 1994). Here we use both analysis of variance and multivariate data analysis to evaluate the response of two Arabidopsis thaliana (L.) Heynh. genotypes to present and predicted future concentrations of ambient CO₂, at low vs. high nutrient supply. We wished to determine if genotypes of A. thaliana known to respond differently to soil nutrients also differed in the amount and/or pattern of their response to CO₂ with emphasis on both single traits and whole plant.

MATERIALS AND METHODS

The genotypes

Two inbred lines (CVI-0 and MS-0) derived from wild populations of A. thaliana were obtained from the Arabidopsis Information Service in Frankfurt, Germany (Kranz and Kirchein, 1987) and then multiplied in controlled greenhouse conditions for two generations. The MS-0 genotype, which is of Capeverde Islands origin, responds more strongly to nutrient addition than CVI-0, which is of Moskun (Russia) origin, by producing big and fast-expanding leafy rosette (Zhang and Lechowicz, 1994). We refer to the MS-0 genotype as more sensitive and the CVI-0 as less sensitive to nutrient availability hereafter.

Experimental procedure

On 4 Jun. 1992, 40 batches of ten seeds randomly selected from each genotype were sown separately in 50 ml centrifuge tubes with a drainage hole at the bottom. The seeds were placed on horticultural sand and covered with about 1 mm of milled peat moss. After sowing, the tubes were soaked in water until the sand and peat moss were saturated. The tubes were then assigned randomly to one of two identical conviron PGW36 growth chambers (20 tubes for each genotype per chamber) with ambient CO₂ set at either present (350) or predicted future (700 μmol mol⁻¹) concentration. Both chambers were set at 23/15 °C day (15 h) and night (9 h) temperatures and 70% relative humidity. Day time light intensity was kept constant at 600 μmol m⁻² s⁻¹ at plant height for both chambers; the number, kind and age of lamps were identical in both chambers. Within each chamber, the 40 tubes were randomized in a space 120 cm long and 40 cm wide at the end of the chamber away from the machine compartment. At 2-d intervals, ten randomly selected tubes for each genotype received 10 ml of 10% and the rest 10 ml of 100% Hoagland solution (version 2. Dunn and Arditti, 1968), yielding (each nutrient addition) 0·25 and 2·5 mg N, 0·05 and 0·5 mg P, and 0·24 and 2·4 mg K, respectively. Water (10 ml) was delivered on alternate days between nutrient addition using a 10 ml auto-pipette. Seedlings were thinned to three as they emerged and then randomly to one per tube within 1 week after the onset of emergence. Ten days after emergence the leaves of each individual were counted, and the length and width of the first true leaf and diameter of the rosette were measured using a pair of calipers. Two weeks later, the same measurements were taken again to calculate the relative increase rate (RIR) of the four variables using the function

\[
\text{RIR} = \frac{(\ln W_f - \ln W_i) / (T_f - T_i)}{W_i} \times \frac{T_f}{T_i},
\]

where \( W_i \) and \( W_f \) are seedling measurements taken at time \( T_i \) and \( T_f \), respectively. Throughout the experiment, plants were monitored daily for the formation of flower buds and individuals were harvested at bolting (start of flower stem elongation). This harvest date was chosen because a previous study (Zhang and Lechowicz, 1994) showed that time of bolting related very closely to all the reproductive events and the final fitness in A. thaliana. At harvest, each plant was cut at the soil surface, leaf number counted, total leaf area measured and the mean chlorophyll content of all the leaves taken using a Minolta dual-wavelength chlorophyll meter (model SPAD-502, Minolta Corp., Ramsey, New Jersey). The SPAD value was converted to chlorophyll concentation following Monje and Bugbee (1992). The roots (which were washed free of sand), leaves and the remaining aboveground parts were dried at 50 °C to constant mass and then weighed. Leaf area ratio (LAR), specific leaf area (SLA) and shoot/root ratio (SRR) were calculated for each individual using the functions

\[
\text{LAR} = \frac{\text{leaf area}}{\text{biomass}}, \quad \text{SLA} = \frac{\text{leaf area}}{\text{leaf dry mass}} \quad \text{and} \quad \text{SRR} = \frac{\text{above ground dry mass}}{\text{below ground dry mass}}.
\]

Data analysis

The data should be analysed using a split plot model that allows for the necessary imposition of the CO₂ treatment at the chamber as opposed to the single plant. However, as is often the case (Hocking and Meyer, 1991a, b; Chu, Coleman and Mooney, 1992; Silvola and Ahlholm, 1992), it was not feasible to use replicate chambers in the CO₂ treatment and hence the CO₂ effects, strictly speaking, should not be directly tested. Nonetheless, we analysed the data as a factorial model on the premise that in this instance the individual plants could justifiably be treated as experimental units; we saw no indication of strong chamber effects and we are confident in our biological interpretation of responses to the CO₂ treatment in this study. The two growth chambers, which were purchased at the same time and have been well maintained, did not differ significantly in environmental condition during the experiment except for ambient CO₂ concentration (22·45 ± 0·005 vs. 22·44 ± 0·002 day and 14·98 ± 0·002 vs. 14·96 ± 0·002 °C night temperature, 69·51 ± 0·006 vs. 69·51 ± 0·007% relative humidity and 352 ± 0·71 vs. 697 ± 0·85 μmol mol⁻¹ CO₂ for chambers set at the present and future CO₂ concentration, respectively). The hour by hour mean temperatures of the two chambers were identical 50% of the time and differed mostly by ±0·1 °C for the rest of the time (Fig. 1). Although the chamber set at the present CO₂ concentration tended to have a slightly higher temperature, the difference was very subtle (±0·1 °C).
Hourly differences between the two chambers in relative humidity were minimal and symmetric about zero difference between chambers. About 350 μmol mol⁻¹ difference in CO₂ was maintained between the two chambers throughout the experiment. Drastic differences between chambers are most likely to be the result of opening doors during measurement and routine management (Fig. 1). Any error introduced by inherent differences between the two chambers is almost certainly negligible compared with the treatment effects themselves and our factorial analysis is reasonable in this instance. The difficulties and expense of replicating CO₂ treatments have led to others adopting the same simplifying assumption (Hocking and Meyer, 1991a, b; Chu et al., 1992; Silvola and Ahlholm, 1992).

All data were screened for errors and three outliers (from different treatments) were excluded. To determine the response of single traits to CO₂ elevation, natural log transformed data were analysed to test the significance of CO₂, nutrient supply, genotype and all the possible interactions for each measured variable (Proc GLM, SAS Inc., 1985). For all the ANOVA models, the three main factors and their two- or three-way interactions were considered as fixed factors.

To gauge the overall response of each genotype to CO₂ enrichment at low and high nutrition, we used canonical discriminant analysis (Proc CANDISC in SAS Inc., 1985), which provides measurements of both the amount and pattern of overall response across all the traits measured. We treated each combination of CO₂, nutrient treatment and genotype as a distinct class. The procedure then generated results showing the weights of each measured trait as well as the class centroid along each canonical axis. The weights of traits or class centroids are assigned in such a way that minimizes within class variance but maximizes variances among classes (Gittins, 1985). Usually, the results are multidimensional in nature as more than two canonical axes (variables) are generated to recover all the information contained in the original data. Often, however, a large proportion of the information contained in the original data can be recovered by the first two or three canonical axes. This allows the presentation of the results in a two or three-dimensional space that is more comprehensible by human imagination. The distance between any two class centroids on the two- or three-dimensional space indicates the overall between-class difference, which is the amount of response to a given factor (CO₂ or nutrient) in our case, and the slope of the line connecting the two class centroids indicates the pattern of response to that same factor. For example, the distance between the class centroids of a genotype under different CO₂ but the same nutrition shows the overall response of that genotype to CO₂ enrichment. Similarly, the distance between the class centroids of a genotype under different nutrition but the same CO₂ concentration indicates the overall response of the genotype to soil nutrient. The relative contribution of each trait to the overall response is indicated by its standardized weights on the canonical axes and the reliability of the analysis can be evaluated by examining the percentage of total variance recovered by these axes. A particular trait may contribute positively or negatively to a given canonical axis, which corresponds to an increasing or decreasing value of that trait as its weight on the axis increases, and vice versa.
RESULTS

Responses in individual traits

Ambient CO₂, soil nutrients and genotype, all had significant effects \((P < 0.05)\) on some of the individual traits (Table 1, Figs 2 and 3). However, variation in plant traits depended more frequently on soil nutrients (12 of 16 traits) and genotype (14 of 16), especially for traits measured at harvest. Only about one third of the traits (6 of 16) were significantly altered by the CO₂ treatment, and these did not include plant biomass which is most closely tied to reproductive success in Arabidopsis (Zhang and Lechowicz, 1994). At \(P = 0.05\), the chance of detecting a significant difference when it is not real is about one out of 16 traits. The differences between 12 out of 16 and 14 out of 16 ex. 6 out of 16 traits are unlikely to result from random variation, suggesting that soil nutrients and genotype are significantly more important factors than CO₂ in determining plant growth.

Significant interactions among the three factors were infrequent (Table 1), suggesting that the two genotypes generally did not differ in single trait responses to CO₂ enrichment. The only significant difference in response to CO₂ elevation between the two genotypes was observed in rate of increase in crown diameter \((P < 0.02)\), which translates into a decrease in rate of increase in crown diameter for the CVI-0 genotype (Fig. 2). For both genotypes, CO₂ enrichment significantly increased seedling leaf length and plant biomass at high soil nutrient but reduced plant biomass at low soil nutrient (Figs 2 and 3). The MS-0 genotype showed greater responses than CVI-0 in rate of increase in crown diameter, area and number of leaves at harvest, chlorophyll content and plant biomass when grown at high nutrient supply (Table 1, Figs 2 and 3).

Responses as a whole plant

The plot of treatment centroids on canonical axes (Fig. 4) provides a comparison of the overall response of all characters to the treatments that complements the univariate analyses. In this study, only the first two canonical axes were used to present the results because they recovered about 90% of the original information (Table 2). Placement of class centroids along axis 1 was mostly attributable to plant biomass, days to bolting (negative loading) and leaf chlorophyll content (negative loading), all of which had standardized weights of high magnitude (Table 2). A group of traits including seedling leaf number and width, seedling leaf length (negative loading) and leaf chlorophyll content had relatively great contributions to axis 2. The MS-0 genotype showed greater overall response to CO₂ enrichment at both the nutrient-poor and -rich conditions, as the (solid)

<table>
<thead>
<tr>
<th>Variables</th>
<th>CO₂(C)</th>
<th>Nutrient(N)</th>
<th>Genotype(G)</th>
<th>C × N</th>
<th>C × G</th>
<th>N × G</th>
<th>C × N × G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf number</td>
<td>2.25</td>
<td>19.77</td>
<td>1.39</td>
<td>1.50</td>
<td>0.01</td>
<td>0.07</td>
<td>2.39</td>
</tr>
<tr>
<td>(10 d)</td>
<td>(0.138)</td>
<td>(0.001)</td>
<td>(0.243)</td>
<td>(0.226)</td>
<td>(0.911)</td>
<td>(0.799)</td>
<td>(0.127)</td>
</tr>
<tr>
<td>Leaf length</td>
<td>17.29</td>
<td>34.74</td>
<td>10.22</td>
<td>5.72</td>
<td>0.03</td>
<td>1.87</td>
<td>4.10</td>
</tr>
<tr>
<td>(10 d)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.002)</td>
<td>(0.020)</td>
<td>(0.860)</td>
<td>(0.176)</td>
<td>(0.047)</td>
</tr>
<tr>
<td>Leaf width</td>
<td>5.02</td>
<td>29.97</td>
<td>0.16</td>
<td>3.87</td>
<td>0.17</td>
<td>0.29</td>
<td>2.12</td>
</tr>
<tr>
<td>(10 d)</td>
<td>(0.028)</td>
<td>(0.001)</td>
<td>(0.053)</td>
<td>(0.078)</td>
<td>(0.394)</td>
<td>(0.150)</td>
<td></td>
</tr>
<tr>
<td>Crown diameter</td>
<td>17.97</td>
<td>27.34</td>
<td>9.66</td>
<td>2.61</td>
<td>2.10</td>
<td>0.29</td>
<td>1.26</td>
</tr>
<tr>
<td>(10 d)</td>
<td>(0.001)</td>
<td>(0.003)</td>
<td>(0.033)</td>
<td>(0.152)</td>
<td>(0.589)</td>
<td>(0.265)</td>
<td></td>
</tr>
<tr>
<td>Leaf number</td>
<td>0.75</td>
<td>9.51</td>
<td>11.69</td>
<td>0.03</td>
<td>1.76</td>
<td>0.26</td>
<td>1.46</td>
</tr>
<tr>
<td>increase rate</td>
<td>(0.389)</td>
<td>(0.003)</td>
<td>(0.018)</td>
<td>(0.059)</td>
<td>(0.189)</td>
<td>(0.609)</td>
<td>(0.231)</td>
</tr>
<tr>
<td>Leaf length</td>
<td>4.54</td>
<td>0.14</td>
<td>24.45</td>
<td>0.92</td>
<td>0.18</td>
<td>0.97</td>
<td>2.77</td>
</tr>
<tr>
<td>increase rate</td>
<td>(0.037)</td>
<td>(0.079)</td>
<td>(0.041)</td>
<td>(0.070)</td>
<td>(0.028)</td>
<td>(0.101)</td>
<td></td>
</tr>
<tr>
<td>Leaf width</td>
<td>3.60</td>
<td>0.12</td>
<td>24.18</td>
<td>0.67</td>
<td>1.47</td>
<td>2.73</td>
<td>0.07</td>
</tr>
<tr>
<td>increase rate</td>
<td>(0.062)</td>
<td>(0.729)</td>
<td>(0.001)</td>
<td>(0.418)</td>
<td>(0.229)</td>
<td>(0.163)</td>
<td>(0.795)</td>
</tr>
<tr>
<td>Crown diameter</td>
<td>1.60</td>
<td>13.10</td>
<td>16.36</td>
<td>0.14</td>
<td>5.96</td>
<td>12.87</td>
<td>0.39</td>
</tr>
<tr>
<td>increase rate</td>
<td>(0.210)</td>
<td>(0.001)</td>
<td>(0.002)</td>
<td>(0.709)</td>
<td>(0.017)</td>
<td>(0.001)</td>
<td>(0.534)</td>
</tr>
<tr>
<td>Days to bolting</td>
<td>8.26</td>
<td>0.30</td>
<td>53.20</td>
<td>2.90</td>
<td>1.30</td>
<td>0.25</td>
<td>3.40</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>(0.005)</td>
<td>(0.586)</td>
<td>(0.001)</td>
<td>(0.093)</td>
<td>(0.258)</td>
<td>(0.619)</td>
<td>(0.070)</td>
</tr>
<tr>
<td>at harvest</td>
<td>1.55</td>
<td>16.53</td>
<td>90.98</td>
<td>2.85</td>
<td>0.25</td>
<td>8.12</td>
<td>4.16</td>
</tr>
<tr>
<td>Area of leaves</td>
<td>(0.218)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.096)</td>
<td>(0.621)</td>
<td>(0.027)</td>
<td>(0.146)</td>
</tr>
<tr>
<td>at harvest</td>
<td>0.12</td>
<td>68.60</td>
<td>51.66</td>
<td>3.56</td>
<td>0.47</td>
<td>26.78</td>
<td>2.35</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>(0.728)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.063)</td>
<td>(0.497)</td>
<td>(0.001)</td>
<td>(0.130)</td>
</tr>
<tr>
<td>Leaf area</td>
<td>0.09</td>
<td>46.73</td>
<td>105.49</td>
<td>3.56</td>
<td>0.00</td>
<td>9.89</td>
<td>2.55</td>
</tr>
<tr>
<td>ratio</td>
<td>(0.759)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.063)</td>
<td>(0.972)</td>
<td>(0.003)</td>
<td>(0.115)</td>
</tr>
<tr>
<td>Specific</td>
<td>3.03</td>
<td>3.36</td>
<td>31.91</td>
<td>1.14</td>
<td>0.14</td>
<td>1.76</td>
<td>0.04</td>
</tr>
<tr>
<td>leaf area</td>
<td>(0.087)</td>
<td>(0.071)</td>
<td>(0.001)</td>
<td>(0.289)</td>
<td>(0.709)</td>
<td>(0.062)</td>
<td>(0.176)</td>
</tr>
<tr>
<td>Shoot/root ratio</td>
<td>0.79</td>
<td>13.86</td>
<td>2.72</td>
<td>0.00</td>
<td>0.07</td>
<td>2.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Biomass</td>
<td>(0.377)</td>
<td>(0.001)</td>
<td>(0.003)</td>
<td>(0.104)</td>
<td>(0.090)</td>
<td>(0.793)</td>
<td>(0.158)</td>
</tr>
<tr>
<td>at harvest</td>
<td>1.60</td>
<td>28.10</td>
<td>71.99</td>
<td>8.07</td>
<td>1.29</td>
<td>17.28</td>
<td>5.39</td>
</tr>
<tr>
<td>(0.211)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.006)</td>
<td>(0.260)</td>
<td>(0.901)</td>
<td>(0.023)</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Bar charts showing mean leaf number (A, B), length (E, F), width (I, J) and crown diameter (M, N) of seedlings and their relative increase rate (RIR) (C, D; G, H; K, L; O, P) for the two genotypes of Arabidopsis thaliana at low vs. high soil nutrients under present and future CO₂ concentration. Vertical bars indicate standard errors. Results of ANOVA are presented in Table 2.

lines connecting the class centroids at the present and future CO₂ concentration are longer at each nutrient supply in MS-0 than in CVI-0 (Fig. 4). Similar results are obtained for the response to soil nutrient (dashed lines) at a given CO₂ concentration. Within each genotype, the MS-0 population exhibited greater overall response to soil nutrient at the future than at present CO₂ concentration. In contrast, the CVI-0 genotype showed a strikingly similar amount of overall responses to CO₂ enrichment at a given nutrient supply or to soil nutrient at a given CO₂ concentration (Fig. 4).

The two genotypes differed not only in the amount but also the pattern of their overall responses to nutrient and CO₂ availabilities. The modest response of the CVI-0 genotype to low vs. high concentrations of CO₂ had the same pattern regardless of nutrient availability (e.g. parallel
solid lines connecting the class centroids). In contrast the pattern of response to CO₂ was distinct at the nutrient-poor vs. nutrient-rich condition for the MS-0 genotype. For example, in response to CO₂ enrichment the MS-0 genotype tended to have increased/decreased values in traits contributed positively/negatively to the first canonical axis (Table 2) at both low and high soil nutrition (e.g. arrows point toward the same direction along axis 1), but decreased/increased values for traits contributing positively/negatively to the second canonical axis only at the nutrient-poor condition (e.g. arrows points downward at nutrient-poor vs. horizontally at nutrient-rich condition). This type of differential shift in traits did not occur in the CVI-0 genotype (Fig. 4).
DISCUSSION

The question asked was if genotypes of *A. thaliana* with different sensitivities to nutrients would respond differently to CO₂ enrichment. We approached the question on the basis of individual traits as well as the whole plant. At the level of single traits, only one (rate of increase in crown diameter) of the 16 measured traits showed significant genotype × CO₂ interaction, while the rest were all far above the 0.05 critical value (Table 1). The results suggest that genotypes of *A. thaliana* with different sensitivities to soil nutrients generally do not vary in their responses to CO₂ enrichment at different nutrient supply. Even though the two genotypes had been exposed to elevated CO₂ for different periods, the trend of increases or decreases in plant traits remained similar. This result differs from other studies showing that the effects of soil nutrients and CO₂ interact with the length of exposure (Benner and Bazzaz, 1988; Arp, 1991). The independence of CO₂ effect on the length of exposure may imply that the growth of plants of the two genotypes were not limited by other factors in this study. Similarly, no significant maternal genotype × CO₂ or maternal genotype × CO₂ × nutrient interactions were observed for various morphological and physiological traits of 1-year-old *Picea mariana* (Mill.) B.S.P. and *Pinus banksiana* Lamb. seedlings (Wang et al., 1994, 1995; Cantin, unpubl. res.). These results emphasize the relatively subtle nature of phenotypic variation between genotypes in response to CO₂ enrichment. Perhaps variation in CO₂ concentration due to global change (Post et al., 1990) or resistance to gas exchange from the bulk atmosphere to individual leaf surfaces (Bazzaz and McConnaughay, 1993) is a much weaker selective force than other environmental factors and thus does not result in differential response of genotypes in individual traits. Intercorelations among traits may also have prevented drastic shift of single traits of plant in response to environmental variation (Schlichting, 1986).

Although the responses to CO₂ in individual traits do not differ between genotypes, this does not mean that the overall response is regulated similarly. Considering the plant as a whole by assessing the combined responses across all the measured traits reveals substantial genotypic differences in both the pattern and amount of plastic response to CO₂ (Fig. 4). As the performance of a plant is determined by the integration of correlated traits, the between-genotype differences in the amount and especially the pattern of overall response to CO₂ enrichment may have significant ecological and evolutionary value. At this whole plant level, the genotype of *A. thaliana* with less sensitivity to nutrient availability also showed less response to CO₂ enrichment, perhaps because the plastic responses to the two factors were coordinated as part of an overall programme for growth and development. Schlichting and Pigliucci (1993) have presented sound arguments for such pleiotropic control through regulatory genes involved in developmental responses to resource availability. Alternatively, the comparable magnitudes of plastic responses to both nutrients and CO₂ may simply follow from genotypic differences in time of flowering; the switch to flowering may truncate the expression of plastic responses in various vegetative traits. In an earlier study, Zhang and Lechowicz (1994) found that the amount of overall plasticity in mature plants of 13 genotypes of *A. thaliana* was positively related to time of flower bud initiation. In this study the late-flowering MS-0 genotype (34 ± 2.58 s.e. d) also was more plastic than the early-flowering CVI-0 genotype (24 ± 0.68) in response to CO₂. Thus, late-flowering genotypes of *Arabidopsis thaliana* tend to be more plastic in response to both soil nutrient (Zhang and Lechowicz, 1994) and ambient CO₂. Functionally, selection for late-flowering genotypes will select concomitantly for increased overall plasticity in response to soil nutrient or CO₂, and *vice versa*. Selection for increased plasticity to soil nutrients will also be associated with increased plasticity to CO₂.

It is interesting that only the more plastic genotype of *A. thaliana* showed changes in the pattern of overall response to different combinations of resource availability. When soil
nutrients are scarce, both nutrient-sensitive and insensitive genotypes may show limited response to CO₂ enrichment due to low leaf nitrogen concentration and photosynthetic capacity (Larigauderie et al., 1988; Ingestad and Agren, 1991). With adequate nutrient supply, however, nutrient-sensitive genotypes may be more responsive to elevated CO₂, perhaps through better ability to balance sink strength between shoot and roots (Ingestad and Agren, 1991), to maintain optimal leaf nitrogen concentration (Hilbert et al., 1991), or to lower the minimum leaf nitrogen for maximum growth (Hocking and Meyer, 1991b). In this study, the overall response of the CVI-0 genotype, which is less sensitive to soil nutrients, to CO₂ remained strikingly similar under both low and high soil nutrition. In contrast, the nutrient-sensitive MS-0 genotype exhibited substantial differences in both the amount and pattern of response to nutrient availability under low and high CO₂. Traits that contributed mostly to the genotype × CO₂ interaction in overall responses did not include rate of increase in crown diameter that showed significant nutrient/genotype × CO₂ interactions according to the test on individual traits (Tables 1 and 2). Perhaps the mechanisms underlying the differences between genotypes in overall response to CO₂ availability involve coordinated phenotypic changes in a variety of functionally inter-related traits. Hocking and Meyer (1991a) found that the response of plant biomass to soil nitrogen was greatly increased by elevated CO₂ in wheat but not in maize. Independent studies also showed little (Hocking and Meyer, 1985) or enhanced (Radoglou and Jarvis, 1992; Wang et al., 1994, 1995) response to soil nutrients by elevated CO₂ depending on species, suggesting that the differences in response to CO₂ across a nutrient gradient may be genetically determined. Furthermore, for a given organism, it is not only the amount of response to CO₂ that may change at low vs. high nutrition but also the pattern of response. For the MS-0 genotype, for example, increasing ambient CO₂ tended to reduce seedling size at low nutrition but increase seedling size at high nutrition (Fig. 2).

To understand the actual impact of future CO₂ enrichment on plants, we need to study the strength of CO₂ effects relative to other abiotic or biotic factors. As shown by this and other studies (Bazzaz, 1990; Bazzaz and McConnaughay, 1992; Wang et al., 1994, 1995), ambient CO₂ is frequently a less strong selective force than other abiotic or biotic factors, such as soil nutrient, moisture, temperature, and competition, at present and probably in the future. Therefore, considering these other factors as major selective forces and then asking how CO₂ enrichment might alter their effects appears to be most relevant (Bazzaz and McConnaughay, 1992; Wang et al., 1994, 1995).

Significant soil nutrient × CO₂ interaction on total biomass was observed in this study, suggesting that the effect of soil nutrients on plant growth and thus final fitness do change with CO₂. For both genotypes, the difference in biomass between plants grown under nutrient rich vs. nutrient poor conditions was increased substantially by CO₂ enrichment, and part of the increment was due to a reduction in biomass of plants grown under nutrient poor conditions with elevated CO₂ (Fig. 3). Plants grown under nutrient rich conditions responded positively to increased CO₂ as commonly observed in many other species (Pearcy and Bjorkman, 1983; Cure, 1985; Chu et al., 1992). The difference in response to CO₂ enrichment between plants grown under the two soil nutrients may have resulted from slightly divergent patterns of biomass partitioning that determines the ability of resource acquisition of the plants (Bhattacharya et al., 1985; Sultan and Bazzaz, 1993). At 350 µmol mol⁻¹ ambient CO₂, plants tended to allocate less dry matter to the shoot and more to the root under nutrient poor as opposed to nutrient rich conditions (Fig. 3), a universally found response of plants to enhance their nutrient uptake ability under low nutrient conditions (Stafford, 1989; Sultan and Bazzaz, 1993). At 700 µmol mol⁻¹ ambient CO₂, however, plants under both nutrient rich and nutrient poor conditions allocated a similar proportion of dry matter to their root systems. Plants grown under nutrient rich conditions also tended to decrease the ratio of shoot-to-root biomass in response to CO₂ enrichment, while those grown under nutrient poor conditions showed the opposite response. Failure of the plants grown under nutrient poor conditions to increase dry matter allocation to root systems in response to CO₂ elevation may have resulted in unbalanced internal resource demands (Bhattacharya et al., 1985; Chu et al., 1992) and consequently reduced biomass at high CO₂. According to this study, CO₂ enrichment tends to reinforce the selective pressure of soil nutrients on A. thaliana genotypes.

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