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# The functional co-ordination of leaf morphology, nitrogen concentration, and gas exchange in 40 wetland species<sup>1</sup>

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**Abstract:** We grew 40 commonly co-occurring species of wetland herbs from eastern North America under uniform conditions to evaluate the overall pattern of interspecific variation in specific leaf mass (SLM), foliar nitrogen content, stomatal conductance ( $g$ ), and internal leaf  $\text{CO}_2$  concentration ( $c_i$ ). While the relationship between any two of these traits that influence net photosynthetic rate is constrained to some degree, there is sufficient flexibility to allow the evolution of different but more or less equally effective interrelationships among these central elements of leaf form and function. We use contemporary techniques of structural equation modelling to describe the general nature of such evolutionary diversification in leaf form and function among these wetland plants. Our model essentially extends the Cowan-Farquhar model of stomatal regulation to include relationships between SLM and foliar nitrogen. The model can take two forms, with variables expressed as either per unit leaf area or per unit leaf mass. When variables are expressed on an areal basis, the model predicts that a species with a higher SLM will have a higher foliar nitrogen level. The foliar nitrogen level, in accordance with the Cowan-Farquhar model, in turn determines the dynamics of stomatal regulation in relation to the marginal cost of water loss relative to carbon gain. The dependence of stomatal regulation on foliar nitrogen also determines the maximal rates of stomatal conductance and net photosynthesis. Internal  $\text{CO}_2$  concentrations within the leaf follow as a necessary consequence of these interrelationships. This areal-based model describes the data for the 35  $\text{C}_3$  wetland species well; the same basic model applies to the five  $\text{C}_4$  species in our sample, except for shifts in the quantitative effects of net photosynthetic rate and stomatal conductance on internal  $\text{CO}_2$  levels. When the variables are expressed on a mass basis, a slightly different model results, as net photosynthetic rate decreases directly with SLM and is not related to species level variation in either leaf nitrogen concentration or maximal stomatal conductance. Both forms of the model indicate the need to advance our understanding of the ecological and evolutionary basis for variation in SLM, including its association with traits such as leaf demography and canopy architecture as well as environmental characteristics of the habitats where particular species predominate.

**Keywords:** comparative ecology, leaf nitrogen, photosynthesis, specific leaf mass, stomatal conductance, path analysis, structural equations modelling.

**Résumé :** Nous avons fait croître 40 espèces de plantes vasculaires communément sympatriques dans les milieux humides de l'est de l'Amérique du Nord sous des conditions uniformes pour évaluer le patron de variation interspécifique de plusieurs variables influençant le taux net de photosynthèse, soit la masse foliaire spécifique (MFS), le contenu foliaire en azote, la conductance stomatique ( $g$ ) et la concentration interne de  $\text{CO}_2$  ( $c_i$ ). Bien que la relation qui existe entre ces caractéristiques, lorsque prises deux à deux, soit quelque peu restreinte, il y a suffisamment de flexibilité pour permettre l'évolution de relations différentes mais plus ou moins également efficaces entre ces composantes importantes de la forme et de la fonction des feuilles. Nous avons utilisé les techniques modernes de modélisation par équations structurales pour décrire la nature générale d'une telle diversification évolutive de la forme et de la fonction des feuilles chez ces espèces de milieux humides. Notre modèle développe le modèle de Cowan-Farquhar de la régulation stomatique pour inclure les relations entre la MFS et le contenu foliaire en azote. Le modèle prend deux formes avec les variables exprimées, soit par unité de surface foliaire ou par unité de masse foliaire. Lorsque les variables sont exprimées par unité de surface foliaire, le modèle prédit qu'une espèce ayant une MFS élevée aura un contenu foliaire en azote élevé. En accord avec le modèle de Cowan-Farquhar, le contenu foliaire en azote détermine la dynamique de la régulation stomatique en relation avec le coût limite de perte d'eau par rapport au gain de carbone. Cette dépendance entre la régulation stomatique et le contenu foliaire en azote détermine également la conductance stomatique maximale et le taux net maximal de photosynthèse. Les concentrations internes en  $\text{CO}_2$  sont la résultante de ces diverses relations. Ce modèle, dont les variables sont exprimées en fonction de la surface foliaire, décrit bien les données recueillies chez 35 espèces de plantes  $\text{C}_3$  de notre groupe expérimental; le même modèle s'applique également aux cinq espèces  $\text{C}_4$  de notre groupe, mises à part quelques différences au niveau des relations quantitatives entre le taux net de photosynthèse et la conductance stomatique et la concentration interne de  $\text{CO}_2$ . Lorsque les variables sont exprimées en fonction de la masse foliaire, un modèle légèrement différent est produit; en effet, le taux net de photosynthèse diminue directement avec la MFS et n'est pas relié au contenu foliaire en azote ou à la conductance stomatique maximale. Les résultats de nos modélisations indiquent qu'il faut améliorer notre compréhension de la base écologique et évolutive de la variation de la MFS et déterminer la relation entre la MFS et d'autres variables dont la démographie foliaire et l'architecture du couvert végétal ainsi que les caractéristiques environnementales des habitats dans lesquels les espèces dominent.

**Mots-clés :** écologie comparative, azote foliaire, photosynthèse, masse foliaire spécifique, conductance stomatique, analyse de pistes, modélisation par équations structurales.

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## Introduction

The necessity of photosynthesis organizes foliar form and function. Specific leaf mass (SLM), foliar nitrogen concentration, stomatal conductance ( $g$ ), and internal leaf  $\text{CO}_2$  concentration ( $c_i$ ) interact to affect net photosynthetic rates ( $A$ ). Although ecophysiolgists recognize functional linkages among these key traits, the nature and consistency of these linkages across species remain uncertain (Cowan & Farquhar, 1977; Field & Mooney, 1986; Reich *et al.*, 1991; Gutschick, 1987; Schulze *et al.*, 1994; Reich *et al.*, 1998; Reich, Ellsworth & Walters, 1997). In any species, such traits vary in response to environmental conditions on time scales from minutes and hours (*i.e.*, stomatal regulation, acclimation) to weeks or even years (*i.e.*, developmental regulation, plasticity).

This lability on physiological and ecological time scales is set against a backdrop of lability on evolutionary time scales. Given this, to what degree are foliar traits free to vary independently in evolution, and to what degree are they constrained by functional considerations? For instance, in a recent analysis of data on hundreds of species, Reich and his colleagues (Reich *et al.*, 1998; Reich, Ellsworth & Walters, 1997) have identified a high degree of convergence in the relationships among  $A_{\text{max}}$ , SLM, and foliar N (Reich *et al.*, 1998) using multiple regression techniques. Multiple regressions are good at determining the predictive ability of a set of predictor variables (SLM and foliar N) relative to a response variable ( $A_{\text{max}}$ ) but they cannot provide falsifiable tests concerning the entire set of direct and indirect relationships between all of the variables simultaneously. In this paper we try to more precisely quantify the interrelationships among foliar morphology and gas-exchange characteristics across species and provide falsifiable tests of such multivariate hypotheses. In particular, (i) what is the entire system of direct and indirect relationships between these traits, and (ii) how strongly constrained is this entire system of interacting traits across species?

Using data from 40 wetland herbs, we develop and test two alternative models describing how the functional linkages in SLM, foliar N,  $g$ ,  $c_i$ , and  $A$  among species are coordinated over evolutionary time. Our analysis is based on the premise that the selective forces that have driven foliar evolution, the physiological mechanisms central to leaf function, and the basic constraints on leaf form, all are sufficiently similar across the species we sampled that broad, interspecific trends will dominate the individualistic responses of each species. It is the nature of these general relationships in the organization of leaf form and function over evolutionary time that we seek to describe in our model. If our working premise is false, then we will not discern any consistent interrelationships in this suite of foliar traits.

Although multiple regression is sometimes used to model suites of interacting traits, this statistical method cannot test such functional hypotheses (Shipley, 1999). Structural equation modelling (SEM: Bollen, 1989; Bentler, 1995), of which path analysis is a special case, is the most powerful method for both quantifying these sorts of interspecific patterns of variation and covariation among traits. More importantly, it is the only statistical technique that can

test, and potentially falsify, such multivariate hypotheses concerning how interacting traits are functionally linked (Shipley, 1999). Unfortunately, this contemporary statistical technique is poorly understood by most ecologists. Structural equation modelling in a biological context is developed and explained in Shipley (2001). Our objectives are not only to develop alternative models describing fundamental relationships among foliar traits, but also to illustrate the use of structural equation modelling in such comparative analyses of form and function.

## DEVELOPMENT OF PATH MODEL 1

The first path model builds on the Cowan-Farquhar model for stomatal regulation (Farquhar & Sharkey, 1982; Farquhar, 1989). We explore the boundary conditions that govern the Cowan-Farquhar model across species. The Cowan-Farquhar model is based on the fundamental trade-off that exists between carbon fixation and water loss in land plants (Figure 1). Since  $\text{CO}_2$  diffusion into the leaf and water diffusion out of the leaf are passive processes that are mediated by stomatal behaviour, the leaf must accept a loss of water in order to affect a gain in carbon. A surprising conclusion of this model is that the leaf should regulate its stomates to restrict carbon fixation below the maximum possible level. This occurs when the internal  $\text{CO}_2$  level in the leaf reaches the "break" point, and the main carboxylating enzyme (RUBISCO) becomes saturated. Further increases in carbon fixation require its regeneration using ATP from the light reaction of photosynthesis. This second stage results in a reduced rate of carbon fixation per unit increase in internal  $\text{CO}_2$  concentration, but the rate of water loss remains at its former level. In other words, although the potential maximum rate of net photosynthesis is determined

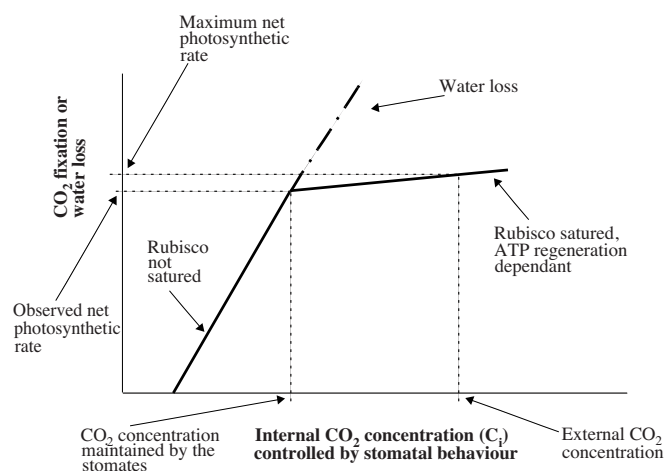


FIGURE 1. Schematic explanation of the Cowan and Farquhar model of stomatal regulation. When stomates are closed, the internal  $\text{CO}_2$  concentration is low and Rubisco is not saturated. As the stomates begin to open, the internal  $\text{CO}_2$  concentration increases and this results in an approximately linear increase in both carbon fixation and water loss. Once the internal  $\text{CO}_2$  concentration reaches the "break point" Rubisco becomes saturated. Further increases in internal  $\text{CO}_2$  due to increasing stomatal conductance will result in only small gains in carbon fixation since this is limited by regeneration of Rubisco. However, water loss increases at the same rate. This results in an increasing cost in water loss relative to the small gain in carbon fixation. Therefore the stomates are regulated to maintain internal  $\text{CO}_2$  at the break point.

by the nitrogen allocated to the photosynthetic machinery independently of the stomatal behaviour, the realized rate of net photosynthesis that is actually observed will be determined by stomatal regulation before this potential is realized. Furthermore, the amount of leaf nitrogen and its partitioning between the light and dark reaction systems will affect stomatal regulation by determining the break point which, in turn, will determine the actual photosynthetic rate. Thus, any relationship between leaf nitrogen concentration and realized net photosynthesis must be indirect. The relative importance of the amount *versus* partitioning of nitrogen will depend on the degree of interspecific variation in amount versus partitioning between the light and dark reactions.

Wullschlegel (1993) compiled estimates of the maximum rates of carboxylation ( $VC_{\max}$ ) and the maximum rates of electron transport ( $J_{\max}$ ) for 109  $C_3$  species, including some data on intraspecific variation in these traits. Although  $VC_{\max}$  varies from 6 to 194 micromoles/ $m^2/s$  (allocation to the dark reaction) and  $J_{\max}$  varies from 17 to 372 micromoles/ $m^2/s$  (allocation to the light reaction), the ratio of the two only varies from 1.1 to 4.6 with 90% of the species having a ratio between 1 and 3. Intraspecific variation was always at least an order of magnitude lower than interspecific variation. Therefore, across species, partitioning is a less important determinant of photosynthesis than the absolute amount of foliar nitrogen. This is consistent with the strong positive correlation between photosynthetic capacity ( $A_{\max}$ ) and foliar N (Field & Mooney, 1986; Reich *et al.*, 1991; 1998; Reich, Walters & Ellsworth, 1997; 1992; Reich, 1993). Reich and his colleagues have further demonstrated the strong dependence of the  $A_{\max}$  to foliar N relationship on SLM (Reich *et al.*, 1998; Reich, Walters & Ellsworth, 1997). These correlational studies are consistent with mechanistic models of the  $A_{\max}$  to SLM relationship (Gutschick & Wiegand, 1988). We therefore identify both foliar N and SLM as critical traits that organize the interrelationships among the variables in the Cowan-Farquhar model when it is applied across species.

We developed a model of the interactions among foliar traits determining photosynthesis (Figure 2a) under conditions in which: *i*) water is not in limited supply, and *ii*) air temperatures, vapour pressures, and  $CO_2$  concentrations are more or less constant when averaged over the lifetime of a leaf. Increasing SLM will result in a larger leaf nitrogen concentration per leaf surface area. The amount and partitioning of leaf nitrogen will determine the dynamics of stomatal regulation that will determine stomatal water conductance,  $CO_2$  influx, and net photosynthetic rates. The increased  $CO_2$  influx will increase internal  $CO_2$  concentration, whereas increased net photosynthetic rates will decrease internal  $CO_2$  concentration. Since water loss is a passive diffusion process, stomatal conductance should be tightly correlated and directly proportional to the number and regulation of the stomates. Stomatal conductance measured at some repeatable reference point therefore will be a good surrogate for the influence of the unknown stomatal regulation mechanism. This composite hypothesis is summarized in a path diagram (Figure 2a) in which "stomatal regulation" is a latent (unmeasured) variable representing the complicated function dictating the response

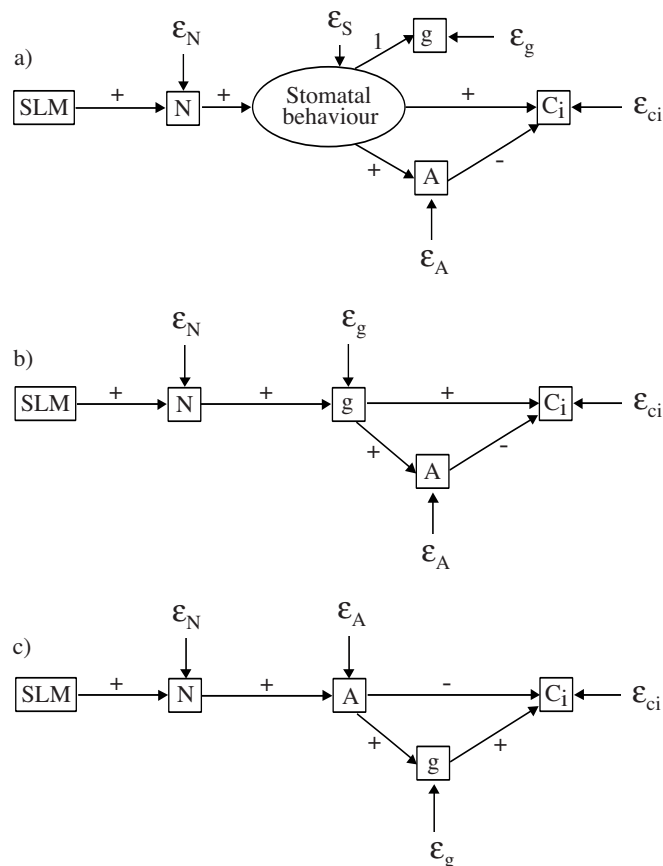


FIGURE 2. Proposed path models. (a) Structural equation model based on the Cowan-Farquhar model under constant environmental conditions. "Stomatal behaviour" is a latent variable and stomatal conductance to water ( $g$ ) is assumed to be directly proportional and highly correlated with this latent. (b) The path model without latents that results from model (a). (c) An alternative model in which it is assumed that the leaf maximizes photosynthetic rate irrespective of water loss. SLM: specific leaf area, N: leaf nitrogen concentration,  $g$ : stomatal conductance to water, A: net photosynthetic rate,  $c_i$ : concentration of  $CO_2$  inside the leaf,  $\epsilon_i$ : residual variance of variable  $i$ .

of stomatal conductance to ambient conditions. We have data on only one cardinal point on this function, which is the stomatal conductance when net photosynthesis is at or near maximum. Assuming that there is a tight and proportional relationship between the overall pattern of stomatal regulation and our measured maximal stomatal conductance, we can define a path model (Figure 2b) involving only measured variables. This path model will only be equivalent to the more general latent variable model derived from the Cowan-Farquhar theory if the correlation between stomatal regulation and maximal stomatal conductance is strong. We tested the model using structural equations techniques (Bollen, 1989; Bentler, 1995) applied to experimental data from a wide variety of co-occurring wetland herbs from eastern North America. The correlation between stomatal regulation and maximal stomatal conductance is likely to be good for the small herbaceous leaves of these species assayed under constant environmental conditions. The resulting set of quantitative linear functions describe the tradeoffs and alternative designs that characterize patterns of ecological and evolutionary diversity in leaf form and function among these wetland herbs.



## DEVELOPMENT OF PATH MODEL 2

The idea that the relationship between leaf nitrogen and realized net photosynthetic rates will be indirect and mediated by stomatal control might seem counterintuitive. An alternative hypothesis is that leaf nitrogen concentration directly determines net photosynthetic concentration and that the stomates are regulated to maximize the net photosynthetic rate irrespective of water loss; this might be especially true in wetland plants. Given this hypothesis, we can then propose an alternative path model (Figure 2c).

These two alternative models predict different patterns of covariation and partial covariation between the measured variables. These differences allow one to test between them and to potentially falsify one or both hypothesized models.

## Material and methods

We compared 40 species (represented by a total of 318 replicate plants) of herbaceous angiosperms that commonly occur on the shorelines of freshwater lakes and rivers in northeastern North America (Table I). The 40 species represent both classes of angiosperms, 6 of the 11 angiosperm sub-classes, 11 orders, 12 families and 28 genera. The subclass Commelinidae (Liliopsida) contained 24 of the 40 species. These were represented only by the orders Cyperales (23 species) and Junciales (1 species); within the Cyperales there were 15 Cyperaceae and 8 Poaceae. This comparison of diverse wetland species, for which few data of this sort otherwise exist (Longstreth, 1989), provides a complement to Field & Mooney's (1986) and Reich's surveys (Reich *et al.*, 1991; Reich, Walters & Ellsworth, 1992; Reich, 1993) all of which are based primarily on upland species, including many from arid or seasonally dry habitats.

## EXPERIMENTAL MEASUREMENT OF PLANT ATTRIBUTES

Seeds were collected from wild plants growing in riverine marshes in southwestern Québec (Canada). Plants were grown from seed in a glasshouse compartment of the McGill University Phytotron. Plants were arranged on the glasshouse bench in randomized blocks. Each plant grew in a separate, freely draining pot in 500 cm<sup>3</sup> of fertile potting soil, supplemented at the beginning of the experiment by a single dose of 20-20-20 fertilizer. Natural lighting, augmented by 1000-watt sodium halide lamps from 06:00 to 20:00, provided 16 hours of light to the plants daily. Day temperatures in the climate-controlled compartment averaged 25°C and night temperatures averaged 20°C. Plants were thoroughly watered daily and automatically misted for 15 seconds every hour during the 16-hour day to maintain wet, but not saturated, soil. These conditions approximate a fertile wetland environment.

We made gas exchange measurements on 3-10 plants per species between 30-40 days after each plant had germinated; Table I gives the exact levels of replication for each species. All plants were assayed under standard conditions in a Conviron PGW36 growth chamber programmed to provide the same photoperiod and temperature regime as in the greenhouse; relative humidity was constant at 70%. The randomly chosen plants to be assayed each day were transferred to the growth chamber 24 hours earlier and each pot, containing a single plant, was placed in a shallow tray

of water. This resulted in soil that was at field capacity so that the gas exchange rates were not limited by water availability. Net photosynthesis, stomatal conductance, and leaf internal CO<sub>2</sub> concentrations were assayed in the growth chamber on one non-senescent, fully-expanded leaf for each plant using a LiCor 6200 Portable Photosynthesis system (Field, Ball & Berry, 1991) with a 1-L cuvette. From one to three consecutive readings were taken for each plant during a two-minute period with the flow of dry air in the cuvette set to minimize the variation in ambient vapour pressure during the assay. All readings were screened for values that exceeded the 95% confidence interval for the species; some outliers were deleted. The subsequent analyses are based on the mean of the replicate readings for each species. While taking the gas exchange measurements inside the growth chamber, workers wore a mask and breathed through a tube leading outside the growth chamber in order to prevent excessive CO<sub>2</sub> enrichment. The median CO<sub>2</sub> concentration inside the leaf cuvette was 439 µL/L, and the 5% and 95% quartiles were 413 and 486 µL/L, respectively. Although these CO<sub>2</sub> levels are higher than those typical of wetlands (approximately 355 µL/L) they are similar to the levels in an urban glasshouse. The median photon flux density at the leaf surface was 636 µmol/m<sup>2</sup>/s with the 5% and 95% quartiles being 508 and 730 µmol/m<sup>2</sup>/s. Under these conditions, the assays provide a good estimate of each species' photosynthetic capacity (A), the associated stomatal conductance (g), and the concentration of CO<sub>2</sub> inside the leaf (c<sub>i</sub>).

Immediately following the gas exchange readings, the part of the leaf that was inside the cuvette was cut out and placed in a plant press. The surface area of each leaf fragment was measured with a DIAS II Image Analyser (Decagon Devices Inc, Pullman, Washington) and its dry mass after 48 hours at 80°C determined. These values allowed us to express net photosynthesis rates and stomatal conductance both on an areal and on a mass basis. Specific leaf mass was calculated from this leaf sub-sample as the ratio of the dry mass to surface area. Note that this estimate of SLM is not strictly equivalent to the traditional whole-plant measurement routinely used in growth analysis, but it is more directly linked to the gas exchange data. After the gas exchange measurements were complete, the entire plant was harvested, the leaf biomass was dried at 80°C in a forced-air oven, bulked by species, and ground through a 40-mesh screen in a Wiley mill. The bulking by species was necessary because a minimum of 0.1 g dry weight of leaves were needed for nitrogen analysis. The ground tissues were analyzed for total Kjeldahl nitrogen using an H<sub>2</sub>SO<sub>4</sub> digestion with potassium sulphate and a selenium catalyst followed by colorimetric assay of the Nesslerized digest (Middleton, 1960).

## STATISTICAL ANALYSIS

The mean values of each variable for each species were used in the interspecific analyses. These mean values, as well as the levels of replication per species, are given in Table I. The variables were first transformed to their natural logarithms to better approximate normal distributions and bivariate linear relationships between the variables. These transformed variables were used to test the path models (Figure 2).

TABLE I. Species means (N[P] = number of readings per species, [number of plants per species] of areal-based and mass-based stomatal conductances (<sup>a</sup>g: mmol/m<sup>2</sup>/s, <sup>m</sup>g: mmol/g/s), net photosynthetic rates (<sup>a</sup>A: mol/m<sup>2</sup>/s, <sup>m</sup>A: mol/g/s) and leaf nitrogen concentrations (<sup>a</sup>N: mmol/m<sup>2</sup>, <sup>m</sup>N: mmol/g), internal leaf CO<sub>2</sub> concentrations (*c<sub>i</sub>*: L/L), and specific leaf mass (SLM: g/m<sup>2</sup>). Taxonomy follows Gleason & Cronquist (1991). Species included in the hypothesized C<sub>4</sub> group are shown with an asterisk (\*)

Species	N[P]	<sup>a</sup> g	<sup>m</sup> g	<sup>a</sup> A	<sup>m</sup> A	<sup>a</sup> N	<sup>m</sup> N	<i>c<sub>i</sub></i>	SLM
<i>Ambrosia artemisiifolia</i>	9[3]	100	2.0	7.97	219.3	81.04	1.65	323	49.12
<i>Artemisia campestris</i>	20[7]	530	4.7	22.09	193.8	231.2	2.03	332	113.96
<i>Bidens cernua</i>	20[8]	120	2.0	5.97	100.5	55.61	0.94	332	59.43
<i>Bidens frondosa</i>	20[8]	120	2.0	5.46	89.2	73.49	1.20	348	61.24
<i>Carex crinita</i>	15[8]	120	3.2	7.35	196.5	57.7	1.54	320	37.40
<i>Carex folliculata</i>	17[8]	70	1.7	5.17	125.4	67.71	1.64	317	41.22
<i>Carex projecta</i>	15[10]	120	4.7	10.7	250.6	73.45	1.71	338	42.85
<i>Carex retrorsa</i>	14[8]	160	3.7	8.1	186.1	57.44	1.32	341	43.47
<i>Carex tuckermanii</i>	18[9]	170	4.3	7.8	203.8	53.06	1.36	344	39.10
<i>Chenopodium polyspermum</i>	11[9]	170	1.0	5.44	31.5	189.00	1.09	363	172.98
<i>Cyperus aristatus</i>	7[6]	320	4.8	21.04	314.0	104.40	1.56	290	67.01
<i>Cyperus diandrus</i>	12[10]	360	4.8	22.82	305.2	103.10	1.38	330	74.78
<i>Digitaria ischaemum</i> *	21[10]	80	1.7	12.11	252.2	38.75	0.81	175	48.01
<i>Dulichium arundinaceum</i>	24[10]	360	5.8	9.97	161.1	168.40	2.72	379	61.87
<i>Echinochloa crus-galli</i> *	21[8]	90	1.9	15.08	316.4	58.89	1.24	157	47.66
<i>Eleocharis erythropoda</i>	17[8]	560	7.9	11.61	164.3	102.00	1.44	382	70.66
<i>Eleocharis obtusa</i>	10[6]	520	9.3	8.55	152.3	73.00	1.30	393	56.13
<i>Eleocharis smallii</i>	13[8]	650	6.7	18.53	192.1	117.80	1.22	383	96.48
<i>Eragrostis pectinacea</i> *	14[9]	240	3.0	20.30	253.8	98.47	1.23	268	80.15
<i>Eupatorium maculatum</i>	22[9]	130	3.3	5.14	128.6	59.40	1.49	354	39.98
<i>Glyceria canadensis</i>	12[8]	440	5.7	13.12	170.7	114.20	1.49	372	76.88
<i>Iris versicolor</i>	23[8]	170	3.6	10.45	222.3	48.68	1.04	318	47.00
<i>Juncus bufonius</i>	5[5]	108	16.5	18.02	276.0	141.8	2.17	381	65.29
<i>Leersia oryzoides</i>	9[5]	150	5.4	6.52	234.4	49.68	1.79	336	27.82
<i>Lycopus americanus</i>	27[10]	150	2.3	9.49	142.6	89.82	1.35	308	66.53
<i>Lythrum salicaria</i>	25[10]	150	1.8	8.21	99.3	83.27	1.01	352	82.68
<i>Panicum capillare</i> *	14[6]	100	1.6	9.35	149.3	63.07	1.01	214	62.62
<i>Phalaris arundinacea</i>	23[9]	150	2.9	9.04	177.5	72.76	1.43	322	50.93
<i>Plantago major</i>	28[9]	130	1.9	4.85	69.5	63.82	0.91	362	69.80
<i>Polygonum lapathifolium</i>	27[8]	180	1.5	11.81	100.6	130.00	1.11	303	117.41
<i>Polygonum pennsylvanicum</i>	16[6]	220	2.6	10.41	121.7	102.60	1.20	347	85.51
<i>Rumex verticillatus</i>	25[9]	200	2.6	7.85	103.6	84.96	1.12	351	75.76
<i>Scirpus americanus</i>	16[8]	2150	14.2	31.00	205.3	294.50	1.95	381	151.03
<i>Scirpus atrovirens</i>	20[9]	260	6.4	11.37	281.7	68.32	1.69	348	40.36
<i>Scirpus cyperinus</i>	10[6]	390	6.1	15.68	247.0	90.68	1.43	350	63.48
<i>Scirpus validus</i>	5[9]	940	7.8	29.40	243.0	205.9	1.70	357	121.12
<i>Sium suave</i>	7[3]	280	4.8	7.44	127.7	99.03	1.70	352	58.25
<i>Spartina alterniflora</i> *	15[8]	300	2.9	26.25	256.8	97.86	0.96	230	102.24
<i>Triadenum fraseri</i>	17[7]	200	4.3	5.29	113.1	71.19	1.52	363	46.79
<i>Xanthium strumarium</i>	16[8]	130	1.0	6.95	55.5	137.70	1.10	338	125.22

#### TESTING OF THE MODEL

The models in Figure 2 are multivariate hypotheses. Because these models are based on interspecific patterns, they must be tested using comparative data. Although such models are quite common in ecology, the testing of such models has often consisted of finding some single statistical prediction of the model and then testing it against the data (for instance, Shipley & Peters, 1990). This is a very weak test since such models make many simultaneous predictions concerning the patterns of correlation and partial correlation among traits, all of which must be observed if the model is indeed correct. A much more powerful statistical test of such models can be obtained through the use of Structural Equations Modelling, or SEM (Bollen, 1989; see Shipley, 1999, for a biological introduction). For instance, the path model in Figure 2b makes predictions concerning 80 different correlations or partial correlations of various orders and all of these predictions are captured in the covariance matrix predicted by the model (Shipley, 1997). The most common statistical test of a structural equation model consists of comparing the covariance matrix predicted by

the model to the measured covariance matrix of the data. After specifying the qualitative relationships among the variables (Figure 2), the maximum likelihood estimates of the parameter values and the resulting predicted covariance matrix were obtained using the EQS program (Bentler, 1995). The null hypothesis is that the observed and predicted covariance matrices are identical except for normally and independently distributed random sampling variation. If this null hypothesis is true then the maximum likelihood chi-squared statistic is asymptotically distributed according to a chi-squared distribution with the appropriate degrees of freedom (Bentler, 1995). Thus, a chi-squared value with a small ( $p < 0.05$ ) probability under the null hypothesis allows us to reject the model. A structural equations model that has a good fit to the data will have a non-significant chi-squared value. This is the reverse of many common lines of statistical inference in ecology (Shipley, 1997).

The statistical assumptions of this method are similar to those of multiple regression, including (i) linear relationships between the variables (perhaps after transformation), and (ii) a multivariate normal distribution for the residuals.

Both of these assumptions are met with our data after logarithmic transformation when using only the 35  $C_3$  species. Because the maximum likelihood statistic only asymptotically follows a true chi-squared distribution, a general rule of thumb is that there be five times as many observations as variables (Bentler, 1995). Since our sample size consists of only 35  $C_3$  species, we used Monte Carlo techniques to estimate the true probabilities of the observed chi-squared values (Bentler, 1995). In other words, we generated 5000 independent multivariate normal data sets based on our fitted models and involving only 35 observations per data set, and determined the empirical 95% quantiles of the maximum likelihood chi-squared statistic. In this paper we report both the Monte Carlo and the asymptotic probabilities (in square parentheses) output by EQS. Analyses of covariance were performed using S-PLUS (Statistical Sciences, 1995).

It is possible to define a basis set of partial correlations that are implied by the two path models (Pearl, 1988). Shipley (2000) has developed an inferential test based on such basis sets that can be used to conduct an alternative method of testing path models. The two alternative path models predict different patterns of partial independence (Table II) and these can be used to test each path model as well as to test between them.

This inferential test (called a d-sep test) is based on the notion of a basis set of (partial) correlations. Every path model makes a series of predictions concerning which correlations or partial correlations between variables must be zero in the statistical population if the model is correct. A basis set is a set of such (partial) correlations that together predicts all others implied by the model. An advantage of this test is that the probability levels are exact, not asymptotic, and can be determined from Spearman non-parametric tests of independence. This allows us to include the remaining five  $C_4$  species in the test. The exact probability levels ( $p_i$ ) associated with each of the  $k = 5$  partial correlations in the basis set of Table II can be combined into the composite test statistic:

$$C = -2 \sum_{i=1}^k \text{Ln}(p_i)$$

TABLE II. Shown are the basis sets implied by the two alternative path models shown in Figure 2. A basis set is a set of independence relationships between the variables in a path model that imply every other independence relationship predicted by the model. These independence relationships can be individually tested by Pearson or Spearman partial correlations and the entire basis set can be tested using the test described in Shipley (2000). The notation " $\rho_{X,Y|Q}$ " means "the partial correlation between variables X and Y, conditioned on a set Q of other variables." Note that the first, second, and fourth partials differ between the two models

Path model 1 derived from the Cowan-Farquhar model	Alternative path model
$\rho_{\text{SLM},g N} = 0$	$\rho_{\text{SLM},A N} = 0$
$\rho_{g,0} = 0$	$\rho_{\text{SLM},g A} = 0$
$\rho_{\text{SLM},Ci \{g,A\}} = 0$	$\rho_{\text{SLM},Ci \{g,A\}} = 0$
$\rho_{N,A \{\text{SLM},g\}} = 0$	$\rho_{N,g \{\text{SLM},A\}} = 0$
$\rho_{N,Ci \{\text{SLM},g,A\}} = 0$	$\rho_{N,Ci \{\text{SLM},g,A\}} = 0$

This test statistic is distributed as a chi-squared variate with  $2k = 10$  degrees of freedom if all of the  $k$  independence relationships are true, as specified by the path model.

## Results

To evaluate the influence of variation in photon flux density, atmospheric temperatures, and  $\text{CO}_2$  concentrations within the leaf cuvette on the gas exchange measurements, we first performed an analysis of covariance on the gas exchange parameters of each leaf. Although these covariates had statistically significant effects, controlling for these effects never changed the species means by more than a single unit. This shows that variation in gas exchange due to between-species variation in environmental conditions was biologically insignificant relative to the phylogenetic variation among this set of wetland species. We therefore report the uncorrected species means for net photosynthesis, stomatal conductance, and internal  $\text{CO}_2$  concentration (Table I).

### BIVARIATE RELATIONSHIPS

Figure 3 shows the scatterplot matrices of the transformed species means expressed on both an areal basis and on a mass basis. These are the data upon which the structural equations models are tested. The scatterplots reveal a set of species that have unusually low  $c_i$  values (squares in Figure 3; cf. Table I). When fitting the path models, we found the normalized residuals of these species were consistently extreme in their contribution to the multivariate kurtosis of the data (Bentler, 1995). Four members of this group, *Echinocloa crus-galli*, *Digitaria ischaemum*, *Panicum capillare* and *Spartina alterniflora* are known to be  $C_4$  species (Elmore & Paul, 1983). The other species (*Eragrostis pectinacea*), although unstudied in this regard, is in a genus known to contain  $C_4$  species (Elmore & Paul, 1983). We therefore removed this set of five known or likely  $C_4$  species from the data used in developing the structural equation models, and we consider their behaviour separately.

The relatively simple bivariate relationships expected among the variables from earlier literature reports generally do occur (Figure 3). For example, the expected positive relationship between foliar N and A is evident in both the areal-based and mass-based data. However, there are also strong correlations between each of these two variables and most of the other variables, indicating complex patterns of covariation as well as the confounding effect of the  $C_3/C_4$  contrast. The scatter in any given bivariate relationship potentially arises in part from interspecific differences in other variables that are covariant with the two variables being considered. The strength of structural equations modelling lies in its ability to determine whether these complicated patterns of covariance could reasonably have been generated by the models specified in Figure 2. Table III lists the bivariate correlations between the five variables, for only the  $C_3$  species, using both the areal- and mass-based standardizations.

### STRUCTURAL RELATIONSHIPS BASED ON THE COWAN-FARQUHAR MODEL

The latent variable model in Figure 2a showed no evidence for any lack of fit to the data involving the 35  $C_3$  species based on the maximum likelihood chi-square ( $\chi^2 = 4.574$ ,  $df = 4$ ,  $p = 0.38$  [0.33]). The path coefficients

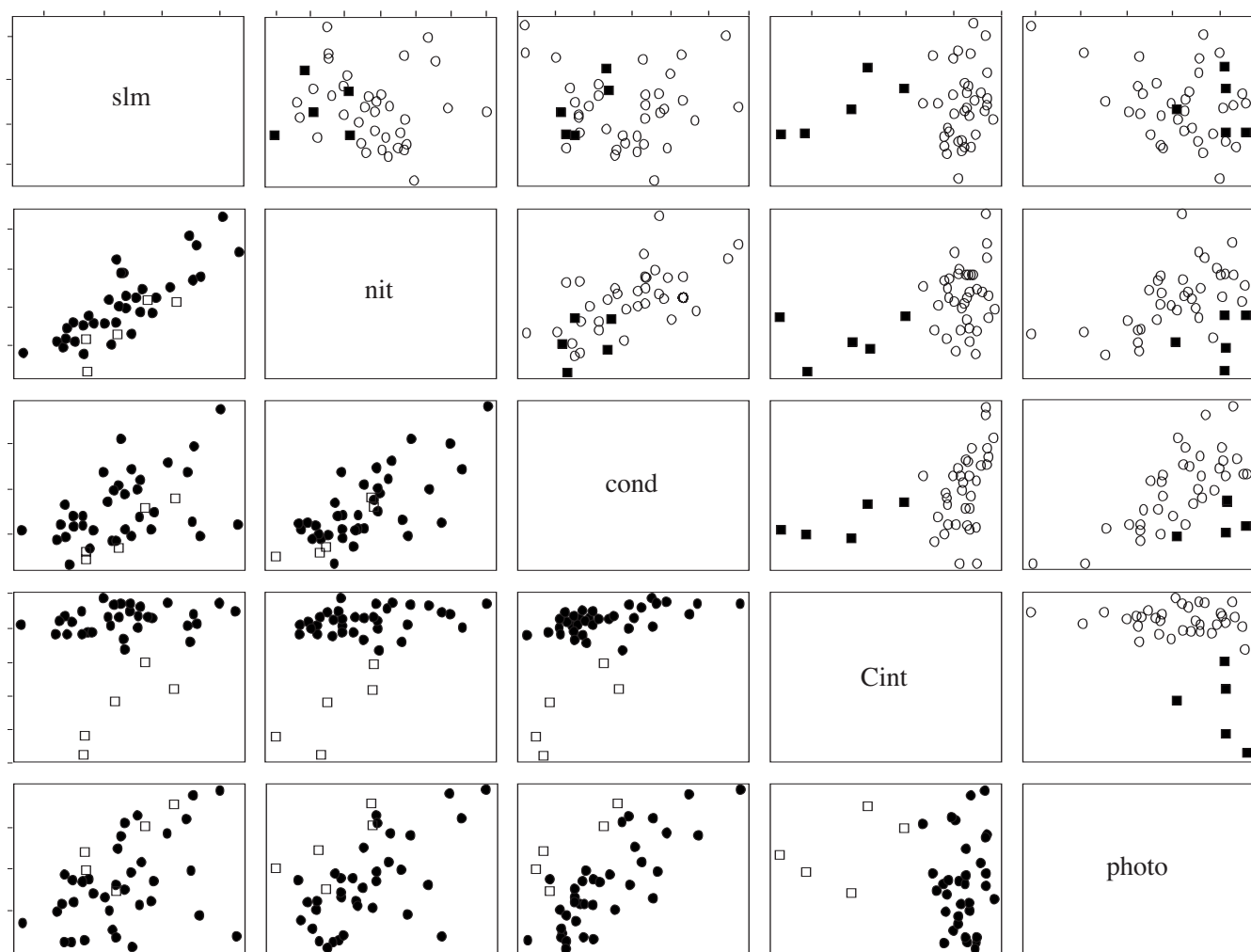


FIGURE 2. Scatterplot matrix of the five quantitative variables included in the path analyses: foliar nitrogen (nit, N), net photosynthesis (photo, A), stomatal conductance to water (cond, g), leaf internal CO<sub>2</sub> concentration (Cint, c<sub>i</sub>), and specific leaf mass (SLM). SLM is always expressed as g/m<sup>2</sup> and c<sub>i</sub> as μL/L. In the upper half matrix the other variables are expressed per unit leaf mass: foliar N as μmol/g, A as μmol/g/s, and g as μmol/g/s; C<sub>3</sub> species are shown as open circles and C<sub>4</sub> species as filled squares. In the lower half matrix the other variables are expressed per unit leaf area: foliar N as μmol/m<sup>2</sup>, A as μmol/m<sup>2</sup>/s and g as μmol/m<sup>2</sup>/s. In all instances the values are transformed to their natural logarithms. Each point is the mean for one of 40 species of herbaceous angiosperms typical of wetland habitats in eastern North America. The abscissa of each graph is the variable name on that row and the ordinate of each graph is the variable name on that column.

based on the standardized variables (with units of standard deviations from the mean) are shown in Figure 4a. The signs of all of the path coefficients are the same as those predicted. The hypothesized path model (Figure 2b) also fits the data well, based on the maximum likelihood chi-square ( $\chi^2 = 4.719$ , df = 5,  $p = 0.50$  [0.45]). Tests of the independence relationships given by the basis set are shown in Table IV; all are consistent with the hypothesized path model and the overall test of the basis set shows no evidence for a lack of fit. This is true for both the 35 C<sub>3</sub> species and for the full data set involving all 40 species when using Spearman (non-parametric) partial correlations. Figure 4b shows this model with the standardized path coefficients, based on the C<sub>3</sub> species. The resulting set of regression equations are listed below (equation 1); all slopes are highly significant ( $p < 0.01$ ).

$$\text{Ln (leaf nitrogen)} = 0.778 + 0.848 \text{ Ln (specific leaf mass)} \quad r = 0.85 \text{ SE}_{\text{RESIDUALS}} = 0.243 \quad [1]$$

$$\text{Ln (stomatal conductance)} = 0.308 + 1.146 \text{ Ln (leaf nitrogen)} \quad r = 0.688 \text{ SE}_{\text{RESIDUALS}} = 0.556 \quad [1b]$$

$$\text{Ln (net photosynthesis)} = -0.708 + 0.548 \text{ Ln (stomatal conductance)} \quad r = 0.807 \text{ SE}_{\text{RESIDUALS}} = 0.30 \quad [1c]$$

$$\text{Ln (internal CO}_2 \text{ concentration)} = 5.436 + 0.142 \text{ Ln (stomatal conductance)} - 0.162 \text{ Ln (net photosynthesis)} \quad r = 0.875 \text{ SE}_{\text{RESIDUALS}} = 0.036 \quad [1d]$$

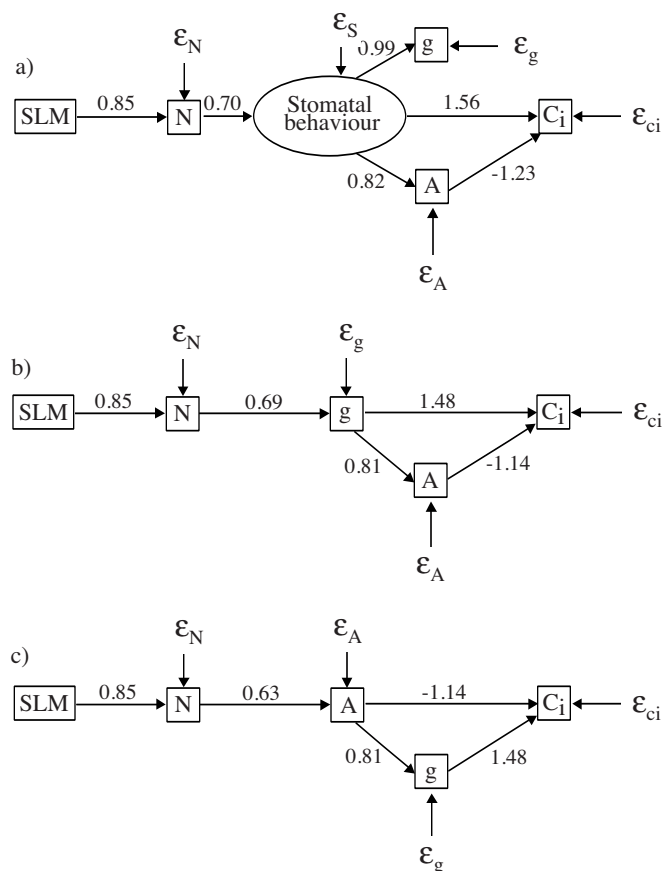
#### THE ALTERNATIVE PATH MODEL

The alternative path model, although not rejected based on the maximum likelihood chi-square and using only the C<sub>3</sub> species ( $\chi^2 = 9.129$ , df = 5,  $p = 0.13$  [0.10]) gives a much poorer fit to the data. When a nested model is fit in which the residuals of net photosynthesis and stomatal conductance are allowed to correlate, a significantly better-



TABLE III. The lower triangle matrix lists the correlation coefficients of the areal-based variables, using the 35  $C_3$  species. The upper triangle matrix lists the correlation coefficients of the mass-based variables

	Specific leaf mass	Leaf nitrogen concentration	Stomatal conductance	Net photosynthesis	Internal $CO_2$ concentration
Specific leaf mass	1	-0.21	-0.11	-0.41	0.21
Leaf nitrogen concentration	0.85	1	0.65	0.6	0.17
Stomatal conductance	0.47	0.69	1	0.74	0.49
Net photosynthesis	0.44	0.63	0.81	1	-0.13
Internal $CO_2$ concentration	0.21	0.29	0.56	0.05	1

FIGURE 4. The same three path models that are presented in Figure 2 except that the standardized path coefficients, obtained from likelihood maximization, are shown. Units of these coefficients are standard deviations from the mean. Note that the correlation between "stomatal behaviour" and stomatal conductance ( $g$ ) is 0.99 in accordance with the assumption of the model.

fitting model results ( $\chi^2 = 3.595$ , 4 df,  $p = 0.51$  [0.46]) showing that there is a significant negative correlation ( $r = -0.55$ ) between these residuals ( $\Delta\chi^2 = 5.534$ , df = 1,  $p = 0.02$  [0.02]). Such a result would be expected if, in fact, net photosynthesis is dependent on stomatal conductance, but the order of the dependent and independent variables was incorrectly reversed. This situation could also arise if there is some unmeasured variable that jointly affects net photosynthetic rate and stomatal conductance, and that is uncorrelated with the other variables in the model. This unmeasured variable would have to increase net photosynthetic rates beyond that predicted by leaf nitrogen content alone and also decrease stomatal conductance below that predicted by net photosynthesis alone.

The exact test, based on the basis set of independence relationships, clearly rejects this alternative model when the  $C_4$  species are included and the tests of independence are based on non-parametric Spearman partial correlations (Table IV). Looking only at the  $C_3$  species, this alternative path model is not rejected, but again gives a much lower probability level than the first path model. Furthermore, the critical partial correlation, predicting the independence of leaf nitrogen concentration and stomatal conductance after controlling for net photosynthetic rate and SLM, is clearly rejected using the both the full data set ( $p = 2 \times 10^{-4}$ ) and using only the  $C_3$  species ( $p = 0.03$ ).

#### MASS-BASED MODELS

The model shown in Figure 2b does not fit the mass-based data well ( $\chi^2 = 11.137$ , df = 5,  $p = 0.069$  [0.049]). Furthermore, the path from SLM to leaf nitrogen is non-significant ( $t = -1.217$ ,  $p = 0.23$ ), contrary to expectations from the model. However, a modification of this model (Figure 5) in which SLM is independent of both leaf nitrogen and stomatal conductance but does directly determine net photosynthetic rate provides a good fit to the data ( $\chi^2 = 3.773$ , df = 5,  $p = 0.62$  [0.58]) with all path coefficients being significant. The predictive equations (equation 6) are:

$$\ln(\text{stomatal conductance}) = 0.72 + 1.74 \ln(\text{leaf nitrogen}) \quad SE_{\text{RESIDUALS}} = 0.52, r = 0.65 \quad [6a]$$

$$\ln(\text{net photosynthesis}) = 5.96 + 0.52 \ln(\text{stomatal conductance}) - 0.39 \ln(\text{specific leaf mass}) \quad SE_{\text{RESIDUALS}} = 0.31, r = 0.80 \quad [6b]$$

$$\ln(\text{internal } CO_2) = 6.43 - 0.15 \ln(\text{net photosynthesis}) + 0.14 \ln(\text{stomatal conductance}) \quad SE_{\text{RESIDUALS}} = 0.04, r = 0.87 \quad [6c]$$

In summary, when gas exchange is expressed per unit leaf mass rather than the more traditional areal standardization, both the SLM and foliar nitrogen investments still have significant influence on the gas exchange characteristics of a species. However (compare Figures 2b and 5) there is now a direct influence of SLM on net photosynthesis (Figure 5), rather than being indirect through its effects on leaf nitrogen and stomatal conductance (Figure 2b). Species with higher SLM simply tend to have lower rates of photosynthesis per unit leaf mass independently of their values of leaf nitrogen and stomatal conductance per unit leaf mass. These latter two variables still have the same effect on net photosynthesis as in the areal model, but they are not themselves affected by variation in SLM.

TABLE IV. D-sep test of the two alternative path models proposed (Figure 2b,c). For each model, the basis set predictions concerning conditional independence relationships of each model are shown; the notation " $\rho_{X,Y|A,B}$ " means the partial correlation between X and Y, conditional of A and B. Next are shown the measured partial correlations and the probabilities of each under the null hypothesis. Two types of partials are shown: Pearson partial correlations were calculated on the subset of 35  $C_3$  species for which normality was obtained and Spearman (non-parametric) partials that were calculated on the entire set of 40 species including the five  $C_4$  species for which normality was not obtained. Finally, the overall test of the entire basis set is shown for each model based on the C statistic, which is distributed as a  $\chi^2$  distribution under the null hypothesis. Values in bold indicate deviations from the null hypothesis significant at the 5% level

Basis set predictions	Path model 1 (Figure 2b)		Basis set predictions	Path model 2 (Figure 2c)	
	$C_3$ species and Pearson partial correlations (probability)	$C_3 + C_4$ species and Spearman partial correlations (probability)		$C_3$ species and Pearson partial correlations	$C_3 + C_4$ species and Spearman partial correlation
$\rho_{SLM,g N} = 0$	-0.298 (0.087)	-0.234 (0.152)	$\rho_{SLM,A N} = 0$	-0.298 (0.087)	-0.234 (0.152)
$\rho_{SLM,A g} = 0$	0.109 (0.539)	0.217 (0.184)	$\rho_{SLM,g A} = 0$	0.221 (0.209)	0.279 (0.085)
$\rho_{SLM,C_i g,A} = 0$	0.043 (0.814)	0.099 (0.556)	$\rho_{SLM,C_i g,A} = 0$	0.043 (0.814)	0.099 (0.556)
$\rho_{N,A SLM,g} = 0$	0.160 (0.374)	-0.069 (0.681)	$\rho_{N,g SLM,A} = 0$	<b>0.371 (0.034)</b>	<b>0.569 (2×10<sup>-4</sup>)</b>
$\rho_{N,C_i SLM,g,A} = 0$	-0.079 (0.668)	0.058 (0.733)	$\rho_{N,C_i SLM,g,A} = 0$	-0.079 (0.668)	0.058 (0.733)
	C = 9.301, df = 10, p = 0.503	C = 9.713, df = 10, p = 0.466		C = 16.00, df = 10, p = 0.100	<b>C = 27.59, df = 10, p = 0.002</b>

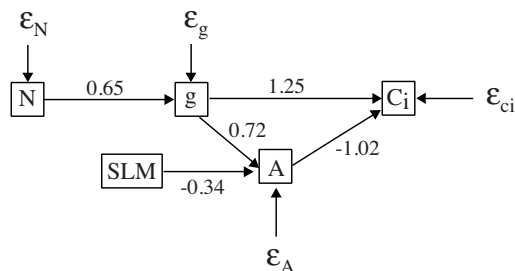


FIGURE 5. An alternative path model for the mass-based variables. Shown are the standardized path coefficients.

## Discussion

The models developed in this study identify the interacting contributions of traits that affect photosynthetic capacity and show that the cost/benefit model of gas exchange developed for an individual leaf (Cowan & Farquhar, 1977; Farquhar & Sharkey, 1982; Farquhar, 1989) can be extended to an interspecific level if coupled with leaf N and SLM. Our models suggest that the uncertainties in a multiple regression model relating photosynthetic capacity to leaf N and SLM (Reich *et al.*, 1998, Reich, Walters & Ellsworth, 1997) might be resolved by explicitly considering: *i*) the influence of  $g$  and  $c_i$ , and *ii*) the covariance relationships among all these traits. For those who are not familiar with structural equations modelling, it is important to emphasize what these results mean and do not mean, both biologically and statistically.

From a biological point of view, the models we have derived illustrate recurrent patterns of covariation in functionally linked traits among different plant species. The relationships among variables in the models describe constraints and patterns in the evolution of leaf form and function that are consistent across species. The individualistic responses of each species around these general patterns (captured in the error variances) are subordinate to the general pattern. These broad, interspecific relationships should not be confused with a mechanistic analysis of relationships among these traits within a given leaf. For

example, the models do not describe the details of stomatal behaviour in a particular species, but only the way that maximal stomatal conductance and photosynthetic capacity are related from species to species. In other words, these interspecific models have their roots in our mechanistic understanding of leaf form and function, but are not themselves ecophysiological models of a leaf.

From a statistical point of view, it is not surprising that one can construct a series of regressions linking these variables related to gas exchange; given the high levels of functional interdependence among these traits almost any two should show a significant correlation within or among species. More importantly, our models define the structure of the relationships not simply between a pair of variables, but among all the interacting variables together. We capture not only the dominant linear relationships between pairs of foliar traits, but also the multivariate, indirect influences of each trait on the overall set of relationships describing the pattern of leaf form and function. Most importantly, we can statistically test and potentially falsify the hypothesized linkages in the entire set of measured variables. It is this ability to describe and test the overall structure of relationships among a set of traits that sets SEM apart from simpler regression analyses more familiar to ecologists. Individual regressions are to structural equation models as individual traits are to phenotypes; just as a phenotype is not a random assemblage of independently evolving traits, a structural equation model is not an arbitrary ordering of separate regressions. A structural equation model must not only predict the values of the dependent trait but must also predict the pattern of covariation among the traits. There are a huge number of different models that could be proposed for the inter-relationships among our five measured variables but the vast majority would fit the data very poorly and be rejected. The strength of SEM lies in our ability to directly express and test alternative models against the overall variance-covariance relationships in the data, not just to rely on multiple regression approaches that ignore partial correlations and covariance among traits.

Structural equation analysis lends itself not only to testing a composite hypothesis derived from present biological understanding, but also to identifying previously unexpected alternative hypotheses worthy of further consideration. In fact, we used the exploratory algorithms of Spirtes, Glymour & Scheines (1993), Spirtes *et al.* (1993), and Shipley (1997) to search for alternative models that provide an acceptable fit on statistical grounds, but such alternative models either contradicted known biological relationships or did not fit the data. The most plausible alternative that we found to our initial composite hypothesis switches the order of stomatal conductance and net photosynthetic rate (Figure 2c). In other words, selection for increasing foliar nitrogen would also act to increase photosynthetic capacity directly rather than through the intermediate influence of selection for increased maximum stomatal conductance. This alternative model has a non-significant  $\chi^2$  when considering only the  $C_3$  species, but is rejected when combining both types of plants and using non-parametric tests of conditional independence. This alternative model seems less convincing on arguments of both parsimony and ecological consistency with the Cowan-Farquhar model of stomatal regulation. The alternative model requires assuming some unmeasured common cause of both stomatal conductance and net photosynthetic rate to account for the significant negative correlation between the residuals of these two variables. It is possible that this unmeasured variable is related to adaptations for water use efficiency, since negatively correlated residuals (in the context of this model) mean that those species whose net photosynthetic rates are higher than predicted based on their leaf nitrogen content also tend to have lower stomatal conductance than predicted based on their net photosynthetic rates. If this were true then this should be reflected in the distribution of these species along the soil moisture gradient that exists in wetlands. However, we see no pattern in these residuals between species that are commonly found in upland or drier soils and those that are always found in wet or submerged soil. Since an alternate explanation for the negatively correlated residuals is simply that of an error in inverting the ordering of stomatal conductance and net photosynthetic rate, and since the models in Figures 2a and 2b are derived from a well-established theory at the level of individual leaves, we prefer this more parsimonious explanation. However, given the low number of species in our study (40) and the resulting low statistical power, the alternative model should not be definitively rejected and should be considered in future research.

There are a number of interesting consequences of our preferred model of interspecific diversification in leaf form and function (Figure 2b). First, the model predicts that the relationship between leaf nitrogen and net photosynthetic rate is not a simple, bivariate relationship, but is dependent on the intervening effects of maximal stomatal conductance (or more likely the overall stomatal regulatory behaviour). Indeed, when conditioned on stomatal conductance, the partial correlation between leaf nitrogen and net photosynthesis decreases from 0.63 to 0.18, which is not significantly different from zero ( $p > 0.3$ ). These results suggest that, despite the significant ability to predict photosynthetic capacity from foliar N (Field & Mooney, 1986) or a

combination of foliar N and SLM (Reich *et al.*, 1998, Reich, Walters & Ellsworth, 1997), fully understanding the basis of interspecific variation in photosynthetic capacity requires direct consideration of stomatal regulation as well.

A second interesting result of the model (Figure 2b) is the emergence of specific leaf mass as a dominant or driving variable in the organization of functional relationships among foliar traits, a possibility also highlighted by recent work of Reich and his colleagues (Reich *et al.*, 1998, Reich, Walters & Ellsworth, 1997). The influence of SLM on foliar nitrogen investments (expressed areally) is unaffected by any of the other variables we have considered: species with greater mass per unit area also have greater nitrogen investment per unit area. This shifts attention to the question of what environmental characteristics or other plant traits are involved in interspecific variation in SLM (Shipley, 1995; Pyankov, Kondratchuk & Shipley, 1999; Meziane & Shipley, 1999). Strong environmental influences on SLM in our data are unlikely, in that all our species grew in a uniform, well-lighted environment. We are not dealing with intraspecific acclimation to sunny *versus* shaded environments, but with interspecific differences in the ways leaves are assembled in a uniform test environment. This interpretation is consistent with the recent demonstration that many leaf characteristics are predictably related to SLM (Reich *et al.*, 1991; 1998; Reich, Walters & Ellsworth, 1997; 1992; Reich, 1993). Variation in SLM is likely to be tightly linked to canopy architecture (Gutschick, 1987; Gutschick & Wiegand, 1988), which changes with habitat light regime (Horn, 1971) and also influences the degree of self-shading. It is reasonable to expect that since a leaf is primarily a carbon-gaining organ, its functional diversification through evolution would be especially contingent on effective light interception. If SLM is adjusted in co-ordination with interspecific variation in canopy architecture, it may be that the traits that underlie interspecific variation in photosynthetic capacity are set at this cascade from canopy- to leaf-scale organization. We need to advance our understanding of the ecological and evolutionary basis for variation in SLM, including its association with traits such as leaf demography and canopy architecture as well as environmental characteristics of the habitats where particular species predominate.

#### AREA- VERSUS MASS-BASED EXPRESSION OF GAS EXCHANGE

One obstacle in modelling leaf or canopy function is the uncertain biological relationship between photosynthesis expressed per unit leaf area versus leaf mass. Although the two measures can be inter-converted using an estimate of SLM, the relationship of photosynthesis to other variables often depends on its expression in areal or mass units and has therefore led to some confusion (Reich, 1993). In this regard, the path model specifying the nature of interspecific relationships among the variables expressed on a mass basis (Figure 5) is of interest. The fact that specific leaf mass no longer determines leaf nitrogen concentration expressed per gram of leaf tissue is to be expected. If areal-based leaf nitrogen concentration depends on specific leaf mass because such leaves have more nitrogen-containing mesophyll per unit area (Konings, 1989), then such a relationship would disappear when nitrogen is expressed per unit leaf



mass. Nonetheless, specific leaf mass does have a (negative) effect of net photosynthesis, expressed per unit leaf mass. Presumably, this reflects the effect of light attenuation through thicker leaf lamellae and therefore the lower photosynthetic efficiency per gram of leaf tissue in those species with thicker leaves.

The mass-based model (Figure 5) also suggests an interesting explanation for the paradoxical result that higher net photosynthetic rates (measured areally) do not generally translate into higher growth rates and yields (Poorter, 1989). Specific leaf mass (the reciprocal of SLA) is often negatively correlated with increased relative growth rates among species (Poorter & Remkes, 1990). An increase in net photosynthesis on an areal basis requires an increased SLM (Figure 3) which, following Poorter & Remkes (1990), will decrease relative growth rates. According to Figure 5, an increased SLM (or decreased SLA) will also decrease net photosynthesis when measured on a mass basis. If a species' relative growth rate is determined by mass-based net photosynthetic rates rather than areal-based photosynthetic rates, then our models are completely consistent with the results of Poorter & Remkes (1990). This suggests that selection for higher mass-based photosynthetic rates may indeed increase growth rates and yield.

### Conclusion

At this stage we view our models as statistical descriptions of the interrelationships among the sampled variables that reflect and are consistent with, but not direct tests of, any mechanistic descriptions of cause and effect. Indeed, the original Cowan and Farquhar model we built upon is not a mechanistic model, but rather a model based on the assumption that leaves have evolved to maximize the cost/benefit relationship between carbon gain and water loss. This restrained interpretation of interspecific path models may be disappointing to some, but it includes the operational meaning that most researchers ascribe to the concept of "ecological strategies" or "functional design," *i.e.*, general interspecific patterns in sets of co-varying attributes determined by selection in a given environmental setting.

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