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Commentary

On the utility of nitrogen in leaves

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Terrestrial environments offer photosynthetic systems of plants several important advantages over conditions in aquatic milieu from which they evolved—larger concentrations of substrates (CO_2 and nutrients) and greater fluxes of photosynthetically active radiation. Those conditions led to a density of vegetation in which competition for light, nutrients, and water became important constraints on evolution. Elevated display of the photosynthetic system in foliar elements is the almost universal solution. That life form entails certain problems, however.

The high evaporative demand of the atmosphere, coupled with a large energy load of absorbed radiation, result in significant loss of water vapor during CO_2 uptake. Heavy, protective cuticles limit gas exchange principally to surfaces with stomatal pores. Combined with water uptake through extensive root systems and efficient transport within the plant through vascular tissues, these traits allow tracheophytes to grow taller, capturing more light, and thereby achieving more photosynthesis than other forms.

Another problem is how best to employ a limited supply of nitrogen. Most plants depend on mineral N (principally NO_3^-) from soil. Reduction of nitrate and synthesis of proteins represent major respiratory costs to plants (1, 2) (symbiotic fixation of N is more costly). Despite continuing mineralization, concentrations of nitrate in soil solutions generally are small (ca. 1–10 μM NO_3^-) because of capture by competitors, immobilization to soil organic matter, and loss through denitrification and leaching. Conversely, the nitrogen requirements of the cumbersome C_3 photosynthetic system are large. Nitrogen in chlorophylls, thylakoid proteins, and associated cofactors and enzymes (particularly rubisco, which may account for 20–40% of a leaf's organic N; ref. 3) comprises about 75% of a leaf's organic N. It is not surprising then that patterns emerge between photosynthetic ability of C_3 leaves and nitrogen contents (refs. 4–7 and others). Light interception by leaves mainly depends on their area and manner of display. Given a finite supply of nitrogen, the evolutionary issue is whether to capture more light with a large area of leaves of small photosynthetic ability or less light with smaller leaves of greater photosynthetic ability.

Reich *et al*. (8) extend to tundra and tropics the pioneering work of Field and Mooney (9), demonstrating general proportionalities across species between maximum net photosynthesis rates (P_n) and leaf properties including organic nitrogen per unit leaf mass, [N]. Leaves of high photosynthetic ability are seen to be thin and short-lived with a large amount of nitrogen per unit mass. Thick, long-lived leaves by contrast have much less nitrogen per unit mass and much smaller photosynthetic ability. Power law aside, P_n increases about linearly with [N] and specific leaf area (SLA, cm^2/g leaf mass), and reciprocally with leaf life span. P_n and [N] thus are reciprocally related to another useful parameter, specific leaf mass (SLM, g mass/cm^2) (9). Reich *et al*.'s data on leaf life span is important for assessing costs and returns of C and N over a leaf's entire life. One might suppose that long-lived leaves with low [N] would provide high nitrogen-use efficiency (P_n/N) but Field and Mooney (9) found that not to be the case.

Although some of the relationships presented by Reich *et al*. (8) involve a degree of autocorrelation, they will prove useful in many ways including analyses of the structure and function of vegetation in biosphere C and N cycles. The relationships are not so simple and perhaps not so meaningful as they might seem, however. Convergence to a common relationship among species requires expressing P_n and nitrogen per unit of dry matter whereas scatter diagrams are obtained when leaf traits of a broad range of species are expressed per unit of leaf area (3, 9). By contrast, clear relationships are obtained within germplasms when nitrogen nutrition is varied and P_n and nitrogen content are expressed on an area basis (refs. 4–7 and others), and those relationships have been a basis for analyzing the nitrogen-use efficiency of crops (10). P_n follows a positive saturation function with nitrogen content and, for crop plants at least, a positive linear function with leaf thickness (11). Furthermore, the ranges of P_n and [N] seen within individual crop species are nearly as great as the ranges presented by Reich *et al*. (8). Field and Mooney (9) found that the area basis also worked well for wild species when analyses were restricted to particular life forms.

The basis for large variation of P_n and [N] within species is that leaf morphology and photosynthetic components are quite sensitive to environmental conditions (irradiance, temperature, and supplies of N and water). Although evolution has brought little variation to the structure of the individual components, their relative proportions differ markedly depending on acclimation that occurs during development and subsequently. Reports by Evans (3, 12), Parkhurst (13), Pearcy and Sims (14), and Terashima and Hikosaka (15) discuss variations in proportions of components and their activities within leaves.

Leaf anatomy is also strongly affected by environment. The number of layers of palisade and parenchyma cells vary little in annual plants (but do vary in trees; ref. 15), but with high irradiance, for example, palisade cells are longer, those of spongy parenchyma are larger (e.g., ref. 16), and lateral expansion of leaves through periclinal divisions (plate meristem activity) is greater. Leaf thickness and SLM increase through an increase in cell size when development takes place at low temperature (17). Limitations by water (e.g., ref. 18) or nitrogen (e.g., ref. 19) result in smaller cells and smaller leaves (fewer periclinal divisions). Mature leaves continue to acclimate to variations in light flux and nitrogen supply through variations in photosynthetic apparatus and [N] (4, 14, 20).

Because Reich *et al*. (8) and Field and Mooney (9) generally measured leaves *in situ* under ambient conditions where the plants had grown, their results confound genetic and environmental effects. Dark reactions of photosynthesis, photorespiration, and maintenance respiration all increase with increasing temperature, and many of their plants may have had only marginal supplies of soil nitrogen. In the work of Reich *et al*., the ranges of P_n, [N], and SLA likely are expanded by acclimation. Sorting that out might require bringing the plants to common environments. Much could be learned, however, through additional measurements on the *in situ* plants. Clar-

ification of the adequacy of water and nitrogen supplies would be important. Subtraction of temporary accumulations of starch, and distinction between nitrogen in photosynthetic structure and the large fraction in nonphotosynthetic components ("other" N), as Evans (3) has done, would be especially valuable. Conversely, expanding the nitrogen base to include all of the productive structure (e.g., stems and petioles involved in display of leaves) would provide another useful index for ecologists.

Leaf cell size is also a ready target for study, and its potential importance can be indicated with spherical cells for which the proportion wall volume/cell volume is a reciprocal function of radius. Although a large portion of the wall volume is occupied by water, wall mass for an "average" cell may constitute 50% of total cell mass. Furthermore, 5–10% of primary wall mass is protein. At 5%, the "average" cell would have 4 mg/g cell mass (0.3 mmol N/g). That amount would account for 50% of the [N] in Reich *et al*.'s low-N leaves. Wall proteins seem to be the main nitrogen fraction remaining in the straws of matured cereals, which generally contain only 5–8 mg N/g mass. This "other" fraction of nitrogen would increase as cell size declined.

The robustness of Farquhar's (21, 22) biochemical model of C_3 photosynthetic systems in dealing with diverse species and conditions indicates that although proportions may vary, like Stein's rose, other attributes of photosynthetic components remain the same. Given the great range of the system's developmental and environmental plasticity, it is clear that the relationships presented by Reich *et al*. (8) are strongly influenced by environment. Until the environmental interactions are unraveled, it is premature to talk about convergent evolution—it may be that only slight divergence from a common source has occurred. Placing biosphere processes on common bases is a critical goal, and relationships between C and N cycles are the central theme. The work by Reich *et al*. will provide a compelling stimulus for continuing research.

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