Plant distribution, stature, rarity, and diversity in a patterned calcareous fen: tests of geochemical and leaf-height models

Laurie A. Stockmeier[1] and Thomas J. Givnish[1,2] (iD)

PREMISE: In patterned fens, patches of short, sparse, species-rich vegetation often occur on substrates rich in precipitated carbonates near calcareous springheads, with taller, denser vegetation farther away. Boyer and Wheeler (1989) hypothesized that phosphorus co-precipitation near springheads limits plant productivity and coverage, and Givnish (1982) proposed that aggregations of rare, short-statured plant species might reflect their competitive restriction to sparsely covered microsites.

METHODS: We tested these hypotheses by quantifying species distributions, leaf heights, plant coverage, community composition, and substrate and leaf chemistry of *Eupatorium perfoliatum* along a gradient of hydrology and geochemistry in a wetland complex in southeastern Wisconsin, USA, ranging from marl flats and fens on peat mounds near springheads to surrounding sedge meadows.

RESULTS: Community composition was strongly correlated with a one-dimensional environmental gradient along which coverage and height increased moving downslope from marl flats, while soil carbonate, phosphorus immobilization capacity, and local species richness decreased, consistent with theory. Regionally rare species were short and restricted to sparsely covered microsites; within and among species, leaf height increased with local coverage. NPK tissue stoichiometry did not entirely support the Boyer-Wheeler hypothesis, although nitrogen limitation appeared strongest in sedge meadows. Shifts in stature and tissue chemistry of *E. perfoliatum* along the marl flat–sedge meadow gradient suggested that zinc toxicity may help limit coverage near springheads despite no significant change in soil zinc content.

CONCLUSIONS: We propose a modified Boyer-Wheeler hypothesis to account for cascading effects of phosphorus co-precipitation near springheads on nitrogen fixation, nitrogen+phosphorus co-limitation, and zinc mobility.

KEY WORDS   *Gentianopsis procera*; geochemical gradients; nutrient limitation; *Parnassia glauca*; rare plants; *Scleria verticillata*; species richness; *Tofieldia glutinosa*; *Trichophorum cespitosum*; *Triglochin maritimum*.

Calcareous spring-fed fens (extremely rich fens, *sensu* Sjörs, 1950) are globally rare and of great interest to conservationists in Europe and North America because they harbor many endangered plant species (Amon et al., 2002; Nekola, 2004; van Diggelen et al., 2006; Jímenez-Alfaro et al., 2012; Seer and Schrautzer, 2014; Duval and Waddington, 2018; Horsáková et al., 2018). Such fens often show internal patterning, with short, sparse, species-rich vegetation near springheads grading into taller, denser, less diverse vegetation on less calcareous substrates (Boyer and Wheeler, 1989; Carpenter, 1995; Bowles et al., 2005). Peat mounds frequently develop under saturated conditions near springheads, resulting in vertical as well as horizontal patterning (Amon et al., 2002; Miner and Ketterling, 2003; Kurtz et al., 2007). The existence of strong geochemical and hydrological gradients within species-rich fens makes them an ideal system for studying the specific conditions that enable rare plants to succeed in certain microsites while failing in others.

Some fen species may be regionally rare partly because they are adapted to the unusual edaphic conditions of wet alkaline peats (Amon et al., 2002; Raney and Leopold, 2018). Many short, rare species may, however, be excluded from more productive sites by

taller competitors, given the likely impact of leaf height on the asymmetric competition for light (Givnish, 1982, 1995). Vojtech et al. (2007) and Emsens et al. (2018) have shown that, in fact, shorter herbs in grasslands and fens are at the expected competitive disadvantage in crowded systems, and Hautier et al. (2009) demonstrated that this disadvantage can be reversed by supplying light at ground level, implicating light competition per se. The open, low nature of calcareous spring fens may thus be a key factor in maintaining populations of regionally rare plants, especially those of relatively short stature. Data from several terrestrial and non-calcareous wetland communities are consistent with the prediction that short-statured species should be relatively rare and restricted to sparsely covered microsites (Givnish, 1982, 1995; Keddy, 1990; Leach and Givnish, 1996; Farnsworth, 2007; Cornwell and Ackerly, 2010). Furthermore, ecological theory and results of field experiments suggest that, in open herbaceous communities, greater plant coverage should reduce local plant species richness (Harpole and Tilman, 2007; Hautier et al., 2009; Borer et al., 2014), based on the competitive impact of leaf height as a function of local coverage first advanced by Givnish (1982). Two central questions regarding fen ecology thus arise: Are rare species in fens generally short in stature and restricted to sparsely covered microsites? And are such microsites especially species-rich?

A second set of issues bears on the cause of sparsely covered microsites within calcareous spring-fed fens. Curtis (1959) argued that such fens in southern Wisconsin, USA, were unstable, succeeding into shrub-dominated communities in the absence of disturbances such as fire, mowing, or grazing. Indeed, certain light-demanding herbs in fens showed competitive release when woody cover was experimentally reduced (Curtis, 1946; Jacobson et al., 1991; Harding, 1993), and native and exotic shrubs invaded several Wisconsin fens coincident with a change in land-use practices. In Europe, mowing, grazing, and burning helped keep some calcareous fens open, and cessation of these practices has led to the local loss of many characteristic species (Opdekamp et al., 2012; Menichino et al., 2016).

By contrast, Boyer and Wheeler (1989) proposed that a geochemical process might result in low productivity and short, sparse vegetation near calcareous springheads in Great Britain. As groundwater percolates through a calcareous aquifer, it dissolves calcium and magnesium carbonates, forming base-rich water with the equivalent (in dissolved carbon dioxide and bicarbonates) of 10 times the current atmospheric concentration of carbon dioxide. When groundwater reaches the surface at or near a springhead the dissolved CO_2 degasses, reducing the solubility of calcium carbonate that, in turn, precipitates near the discharge point. Boyer and Wheeler (1989) argued that this process results in the co-precipitation of phosphorus in an insoluble form, leading to reduced P availability to plants. Consistent with this hypothesis, they found significantly more calcite precipitated in the substrate under the short, sparse vegetation near springheads in British fens than in the taller surrounding vegetation. Experimental fertilization of a phytometer species grown in peat collected from patterned fens showed that P—not N or K—limited plant growth in short fens, but not in tall fens. In intermediate to rich fens in Belgium, Boeye et al. (1997) found P limitation in microsites with low standing crops, and N limitation in microsites with high standing crops. P limitation has subsequently been demonstrated in several calcareous spring fens via fertilization experiments or inferred from NPK stoichiometry of live leaf tissue (e.g., Koerselman and Meuleman, 1996; Bedford et al., 1999; Olde Venterink et al., 2003; Rozbrojová and

Hájek, 2008), but some fens appear to be jointly limited by N and P (Cusell et al., 2014; Øien et al., 2018), and sulfide production plays a key role in reducing coverage by dominant plants near springheads in one New York fen (Simkin et al., 2013).

Changes in fen vegetation over the past few decades have raised conservation concerns in Europe and North America (Verhoeven et al., 1988; Jacobson et al., 1991; Fojt, 1994; van Diggelen et al., 1996, 2006; Jímenez-Alfaro et al., 2018) and have been variously ascribed to eutrophication, invasive species, or changes in hydrology or disturbance regime. The relative importance of hydrology and traditional management activities (mowing, grazing, burning, turf cutting) has been much debated, especially in Europe (Fojt and Harding, 1995; Opdekamp et al., 2012). Boyer and Wheeler's geochemical mechanism, if correct, suggests a means of managing fens that would depend critically on the maintenance of regional hydrology, with central importance attached to sustaining high flow rates of carbonate-laden water from springheads.

Here, we quantify the distributions, stature, and diversity of vascular plant species, soil chemistry, and NPK stoichiometry of *Eupatorium perfoliatum* within the Clover Valley wetland complex in southeastern Wisconsin, including steep local gradients in coverage from several springheads into surrounding sedge meadows. We use these data to test (1) whether rare species are relatively short-statured and restricted to zones of sparse plant coverage; (2) whether such zones are unusually diverse, with species richness decreasing toward more productive microsites; and (3) whether the Boyer-Wheeler model for P limitation causing sparse coverage near springheads appears to be valid at Clover Valley. Based on our findings, we advance a modified version of the Boyer-Wheeler model, including putative effects of P immobilization near springheads on soil N fixation and N co-limitation, as well as on zinc mobility, and the potential that Zn toxicity helps drive low coverage and high plant diversity near springheads.

MATERIALS AND METHODS

Study site

Clover Valley Scientific Area (42°47′N, 88°42′W) consists of fens and related vegetation on several peat mounds (1–3 m tall) scattered in a matrix of sedge meadows occupying a former glacial lakebed. This 26.6 ha wetland lies at the base of the Kettle Moraine, near Whitewater in southeastern Wisconsin. Rising 30 m above the surrounding landscape, this moraine is composed mainly of dolomitic till, derived from the Niagara Formation and deposited during the Wisconsinan glacial advance. The peat mounds clustered in this wetland complex afford an opportunity to study replicate community gradients in a compact area, in which dispersal limitations are unlikely to have a strong effect on species distributions. Because these mounds differ somewhat in height, vegetation type, and presence of spring-fed pools, they may also encompass a primary successional sequence. Some of the smaller mounds have open spring-fed pools at their summits, while the larger mounds have less obvious seepage zones on their flanks.

Stratigraphic and hydrologic analyses of nearby peat mounds have shown that fine lacustrine sediments (clays and silts) present in the basin are absent or very thin beneath the mounds (Ciolkosz, 1965; Kratz et al., 1981). These clayey sediments—the remains of a glacial lakebed—confine groundwater infiltrating from the moraine

uphill, with upward artesian flow occurring wherever the lakebed is breached. Peat accumulates under anoxic, saturated conditions across the poorly drained glacial lakebeds, forming mounds under the constantly saturated conditions at the springheads. Water levels in the poorly drained sedge meadows downslope vary, with standing water present in spring and late fall, but not between rains in the summer.

Three intergrading vegetation types, based on stature and composition, occur at Clover Valley: (1) *marl flats* are characterized by small patches of short, sparse turf mostly <20 cm tall in autumn, dominated by the sedges *Rhynchospora capillacea* and *Scleria verticillata* and overlying a substrate rich in precipitated carbonate (tufa); (2) *fens* (*sensu* Carpenter, 1995) are dominated by grasses, sedges, and various forbs <65 cm tall in autumn, occurring on peat with or without tufa; and (3) *sedge meadows* (*sensu* Curtis, 1959) are dominated by the tussock-forming *Carex stricta*, several grasses, and a few tall Asteraceae, generally >65 cm tall in autumn and overlying a peat or muck substrate. Indicator species for these three informal vegetation types (Table 1) were identified during reconnaissance and used to guide stratified sampling.

Despite limited grazing a few decades ago, coverage by introduced weeds is very sparse. The study site harbors six vascular plant species considered rare in Wisconsin, including two state-threatened species (*Trichophorum cespitosum* [Cyperaceae] and *Triantha glutinosa* [Tofieldiaceae]), two species of special concern (*Scleria verticillata* [Cyperaceae] and *Triglochin palustris* [Juncaginaceae]), and two species recently removed from the special concern list (*Gentianopsis procera* [Gentianaceae] and *Solidago ohioensis* [Asteraceae]).

Vegetation sampling

Sixteen transects were established, radiating from six zones of marl flats or fens on four peat mounds into the surrounding matrix of sedge meadow, along a gradient of increasing plant stature and coverage (Appendix S1). Transects were located to avoid areas dominated by large shrubs. To stratify sampling among all three a priori vegetation types, we divided each transect into two or three segments, depending on the number of vegetation types crossed. Each segment was subdivided into four or six reaches of equal length, and a total of 12 permanently marked quadrats (0.5 × 0.5 m) were then established along each transect in a stratified random spatial array. Coverage of each vascular plant species in each quadrat was visually estimated, and maximum leaf height measured, during late June, late July, and mid-September, during the early, middle, and late growing season. Given the difficulty of distinguishing vegetative

culms of individual species in *Carex* and Poaceae, estimates were simply made of total coverages by *Carex* spp. and by grasses.

Environmental measurements

As an index of local vegetation stature, a visual estimate was made of the height below which 90% of the biomass occurred in each quadrat. The leaf area index (LAI: m^2 leaf area/m^2 ground) of total plant cover in each quadrat was estimated using a ceptometer (AccuPAR LP-80: Decagon Devices, Pullman, Washington, USA), based on measurements of photosynthetically active radiation (PAR: $\mu mol\ m^{-2}\ s^{-1}$ 400–700 nm) above and below canopy during the July sampling, and assuming a spherical leaf-angle distribution.

The relative elevations of quadrats along each transect were measured by running a profile with a clinometer and surveyor's rod. We then calculated a relative slope position for each quadrat between 0 and 1, treating each transect separately. Piezometers were not installed because of the danger of piercing the clay barrier to artesian flow.

After vegetation sampling was completed, peat cores (2 × 3 × 10 cm deep) were collected from the center of each quadrat using a sharp knife and refrigerated at 4°C for up to a few days. Peat samples were dried at 70°C to constant mass in a forced-air oven and then cooled in a desiccator. Cores were ground with a mortar and pestle and passed through a 1.5 mm mesh sieve. Subsamples (1.0 g) were weighed into crucibles and incinerated in a muffle furnace in a two-step procedure, in which loss on ignition at 550°C was used to determine organic content and loss at 1000°C was used to determine carbonate content (Dean, 1974).

Phosphorus immobilization capacity (PIC) of each core was determined by adding 25 mL of a KH_2PO_4 solution (10 ppm P) to a 1.00 g subsample of dried, pulverized, and screened peat in an Erlenmeyer flask. Samples were agitated on a reciprocating shaker for 1 h, allowed to sit for 23 h, then shaken again for 10 min (Nieminen and Jarva, 1996). Extracts were immediately filtered through no. 2 Whatman paper and refrigerated until analysis. Phosphate concentration was determined by the Murphy-Riley colorimetric method (Murphy and Riley, 1962). A subset of the prepared peat samples was submitted to the University of Wisconsin Soil and Plant Analysis Laboratory for digestion with nitric and perchloric acid and analysis for total P, K, and various micronutrients by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Phytometer study

To provide insight into the actual availability of P and other elements to plants along the stature/productivity gradient, we measured variation in the elemental composition of leaf material in *E. perfoliatum*, as well as its allocation of dry mass to leaves, stems, and reproductive structures. This species was chosen as a phytometer because it occurs across the entire vegetational gradient. None of the initial transects had enough *E. perfoliatum* for study, so we established a separate transect in an area where it was common. This transect was divided into marl-flat, fen, and sedge-meadow segments; each segment was divided into six equal reaches, and a sampling point was located along each reach using a random-numbers table. We selected the flowering individual of *E. perfoliatum* closest to each point, measured its maximum leaf

TABLE 1. Indicator species used to categorize vegetation zones and stratify sampling at Clover Valley Scientific Area, Wisconsin, USA.

Vegetation type	Indicator species
Marl flat	*Lobelia kalmia, Rhychospora capillacea, Scleria verticillata, Solidago ohioensis, Triglochin maritimum*
Fen	*Andropogon gerardii, Cirsium muticum, Lysimachia quadriflora, Muhlenbergia glomerata, Parnassia glauca, Sorghastrum nutrants, Valeriana edulis* var. *ciliata*
Sedge meadow	*Carex stricta, Eutochium maculatum, Solidago gigantea, Symphyotrichum firmum*

height, harvested its aboveground portion, and collected a peat core from its base.

Tissue samples were taken immediately to the laboratory, where stem, leaf, and reproductive tissues were separated, dried at 70°C to a constant weight, and weighed to determine biomass allocation. Dried leaf tissue was ground using a Wiley mill with a 60 mesh screen and sent to the University of Wisconsin Soil and Plant Analysis Laboratory to quantify N content by the Kjeldahl method, and P, K, and micronutrients by ICP-OES (see above).

Data analysis

Indirect gradient analysis—For each species, abundance in each quadrat was quantified as the maximum coverage achieved across the three censuses. Species coverages were then relativized by the total of such coverages across all taxa occurring in a quadrat. Based on relative species coverages, we ordinated quadrats in three abstract dimensions, using both Bray-Curtis (BC) ordination and non-metric multidimensional scaling (NMS) as implemented in PC-ORD (McCune and Mefford, 1997). Ten NMS ordinations were executed with random starting points; an additional run (NMS/BC) was conducted using the results of the BC ordination as a starting point. We then overlaid the abundances of several representative species on each ordination and calculated the correlations of various environmental measures with ordination-axis scores. We assigned quadrats a posteriori to vegetation types based on the average axis 1 score of indicator species (Table 1), weighted by their maximum seasonal coverage in each quadrat.

Initial observations suggested that the substrate often showed substantial heterogeneity at small spatial scales, with subsurface veins of tufa penetrating the peat. To assess the impact of such small-scale heterogeneity, we conducted correlations and regressions of axis scores on peat chemistry using both raw and smoothed data. To smooth data, we ranked samples by carbonate content (or PIC) and then grouped them into non-overlapping subsets of 10 consecutive samples. We averaged the values of carbonate content (or PIC) and of various environmental characteristics for each subset and then employed subset means in subsequent statistical analyses.

Leaf height—After eliminating species that occurred in fewer than five quadrats (<2% frequency), we used least mean squares linear regression to test whether mean maximum leaf height (measured during the September census) increased significantly with the mean July LAI and mean July coverage in which individual species occurred. To examine within-species variation in leaf height along coverage gradients, we selected four common species that were widespread on the study transects (*Galium boreale*, *Gentianopsis procera*, *Lysimachia quadriflora*, *Pycnanthemum virginianum*) and regressed their maximum leaf height within quadrats against quadrat LAI.

To test the hypothesis that rare species are shorter and restricted to more sparsely covered microsites than common species, we compared the mean leaf height of rare species ($n = 6$) and all other species ($n = 40$) using a one-tailed *t*-test, assuming unequal variances. The same procedure was used to compare the mean LAI in which rare and common species are found.

Species richness—We used linear regression to test whether quadrat species richness (number of taxa per 0.25 m²) decreased with LAI, as expected. "Species richness" here refers primarily to forb species richness, given that grass species and sedge species were reduced to a single taxon each. Backward-elimination and forward-addition multiple regression were also employed to identify additional environmental measures at the quadrat level that were the best predictors of quadrat species richness. We also related species richness to axis 1 scores from the ordination.

Phytometer study—Pairwise comparisons of maximum leaf height, biomass allocation, and leaf nutrient concentrations for individuals in different vegetation zones were performed using two-tailed *t*-tests assuming equal variances. Backward-elimination multiple regression was used to select the tissue nutrient concentrations that best predicted leaf height.

To assess nutrient limitation by tissue stoichiometry, leaf N:P ratios were first compared with those for plants in freshwater wetlands whose productivity had been shown experimentally to be N limited (N:P < 14), P limited (N:P > 16), or N and P co-limited (14 < N:P < 16) (Koerselman and Meuleman, 1996). Similar tests were conducted using NPK stoichiometry using equations derived by Olde Ventertink et al. (2003). Here, sites limited by N are characterized by N:P < 14.5 and N:K < 2.1; sites limited by P or co-limited by P and N are characterized by N:P > 14.5 and K:P > 3.4; and sites limited by K or co-limited by K and N are characterized by N:K > 2.1 and K:P < 3.4. Pairwise comparisons of the mineral composition of peat collected from the base of each specimen were conducted in like manner. These data were combined with measurements of peat chemistry for a subset of the samples collected in the vegetation study and together were analyzed for significant differences in substrate chemistry among zones.

RESULTS

We found a total of 83 herbaceous species in 191 quadrats (each 0.25 m²) sampled; seven more species grew near the study transects. Among all species, 63 were forbs (accounting for 59% of total maximum cover) and 27 were graminoids. The number of graminoid species was underestimated because grasses were combined into a single category for cover estimation and because several nonreproductive sedges were not identified. Based on the a priori classification of quadrats into vegetation types based on indicator species, 45 quadrats occurred in marl flats, 72 in fens, and 74 in sedge meadows.

Indirect gradient analysis

Bray-Curtis, NMS, and NMS/BC ordinations all yielded qualitatively similar results in the relative placement of quadrats corresponding to each vegetation type and relationships of axis scores to measures of the physical environment. We thus present only the results of the NMS/BC ordination, which explained the greatest proportion of variation in species composition.

The NMS/BC ordination showed no apparent discontinuity in vegetation composition (Fig. 1), despite the occasional occurrence of abrupt ecotones in the field. We rotated the ordination to maximize the correlation of axis 1 scores with ln (LAI), the environmental parameter showing the strongest relationship to the ordination axes. After rotation, axis 1 accounted for 46% of the variation in species composition, axis 2 for an additional 18%, and axis 3 for a further 14%.
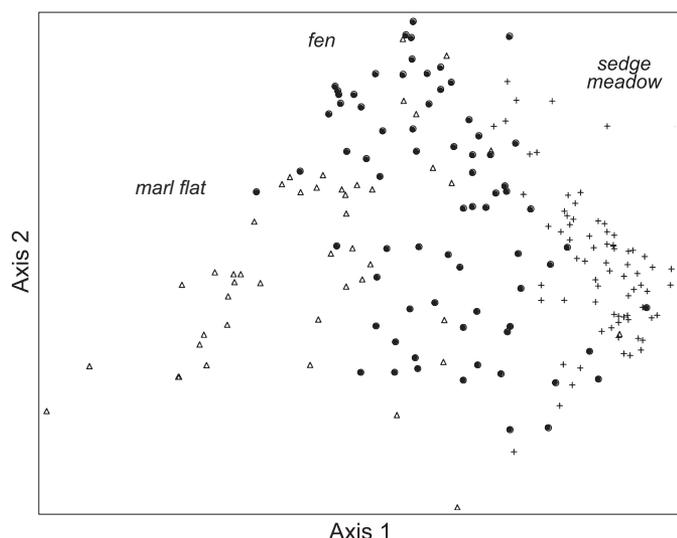
FIGURE 1. NMS/BC ordination of 191 quadrats and 72 species at Clover Valley Scientific Area, Wisconsin, USA. A posteriori vegetation classification of quadrats is indicated by symbols: triangles = marl flat; filled circles = fen; plus signs = sedge meadow.

Only axis 1 showed strong correlations with any quantified measure of the physical environment (Fig. 1; Table 2); those measures were themselves strongly cross-correlated (Appendix S2). Axis 1 scores increased strongly with vegetation height and various indices of coverage: LAI, ln LAI, and summed coverage in June, June, and September (Table 2). The positive correlation of axis 1 scores with vegetation height increased as the growing season progressed, reflecting the seasonal increase in the range of plant heights across quadrats. Axis 1 scores declined toward higher slope positions, and—more weakly, at the level of individual quadrats—with carbonate content and PIC (Table 2). Data smoothing, however, greatly increased the correlation of axis 1 scores with both carbonate content and PIC (Table 2), so that both of these factors and slope position

TABLE 2. Environmental correlations (r) with NMS/BC ordination axes in this study at Clover Valley Scientific Area, Wisconsin, USA. Axis 1 scores explained an average of 40.1 ± 20.2% of the variance in the individual environmental measures shown, significantly more than the 5.8 ± 6.8% explained by axis 2 or the 1.5 ± 3.0% explained by axis 3 (two-tailed paired t-tests, df = 14, $P < 0.0001$).

Environmental measure	Axis 1	Axis 2	Axis 3
Organic matter (%)	0.204	−0.008	−0.087
Carbonate (%)	−0.480	0.028	0.029
Smoothed % carbonate	−0.837	0.177	0.077
Smoothed ln (% carbonate)	−0.840	0.154	0.138
Leaf area index (LAI)	0.717	−0.190	−0.008
ln (LAI)	0.777	−0.096	0.080
Vegetation stature (cm)—early	0.655	−0.158	0.026
Vegetation stature (cm)—midseason	0.685	−0.224	−0.046
Vegetation stature (cm)—late	0.710	−0.295	−0.083
Total cover (%)—early	0.508	−0.347	0.178
Total cover (%)—midseason	0.558	−0.467	−0.040
Total cover (%)—late	0.575	−0.427	−0.046
Phosphorus immobilization capacity (PIC)	−0.225	0.016	0.133
Smoothed PIC	−0.649	0.096	0.343
Relative slope position	−0.662	0.277	−0.052

decreased sharply along axis 1 while vegetation height and various measures of coverage increased, reflecting the correlation structure of these environmental measures with each other (Appendix S2). Axis 1 scores explained an average of 40.1 ± 20.2% of the variance in the individual environmental measures quantified, significantly more than the 5.8 ± 6.8% explained by axis 2 or the 1.5 ± 3.0% explained by axis 3 (two-tailed paired t-tests, df = 14, $P < 0.0001$). Axis 2 scores declined modestly with July and September coverages; axis 3 scores increased modestly with smoothed PIC (Table 2).

A posteriori assignment of quadrats to vegetation types based on the average axis 1 score of indicator species, weighted by species coverage, identified a gradient from marl flats through fens to sedge meadows running primarily across axis 1 (Fig. 1). The distribution of vegetation types scored a priori (not shown) was nearly identical. Average differences between a posteriori vegetation types in several environmental measures (Appendix S3) agreed with the trends expected based on the correlations of those measures with axis 1 scores. That is, vegetation height, coverage, and midseason LAI increased from marl flats to fens, and from fens to sedge meadows, whereas peat carbonate content, PIC, and slope position declined. Soil organic matter decreased from marl flats to sedge meadows, presumably reflecting the increase in carbonate content (Appendix S3). Detailed analyses of substrate chemistry showed that P, K, copper (Cu), and especially Fe increased significantly in concentration from marl flats to sedge meadows, while B and S declined significantly (Table 3). Mn showed a substantial but statistically nonsignificant, nearly 50% increase in concentration toward sedge meadows.

Leaf height

Average maximum leaf height varied from 6.4 cm in *Parnassia glauca* to 99.5 cm in *Typha latifolia*. Overlays of the abundance of six representative species of different height on the NMS/BC ordination showed that the short species (*P. glauca*, *Agalinis purpurea*) reached peak abundance in marl flats; taller species (*Solidago riddellii*, *Pycnanthemum virgianum*) tended to reached peak abundance in fens, in the middle of the coverage gradient; and the tallest species (*Lysimachia quadriflora*, *Symphyotrichum firmum*) reached peak abundance in sedge meadows, in the most densely covered site (Fig. 2). Of the six regionally rare species included in our study, three (*Scleria verticillata*, *Triantha glutinosa*, *Triglochin palustris*) were mostly restricted to marl flats, while the other three were

TABLE 3. Mean (± SD) concentrations in parts per million of various nutrients in 10 peat samples in each of the three vegetation zones at Clover Valley Scientific Area, Wisconsin, USA. Significant differences (pairwise two-tailed t-tests, df = 18, $P < 0.05$) are indicated by different superscript letters.

Nutrient	Marl flat	Fen	Sedge meadow
Phosphorus	1050 ± 234[a]	1280 ± 144[b]	1690 ± 239[c]
Potassium	642 ± 151[a]	775 ± 166[ab]	793 ± 152[b]
Calcium	88,500 ± 40,500[a]	60,700 ± 22,400[a]	64,200 ± 23,800[a]
Magnesium	6380 ± 1140[a]	6090 ± 829[a]	5630 ± 733[a]
Sulfur	5590 ± 1660[ab]	5740 ± 1060[a]	4480 ± 756[b]
Zinc	52.0 ± 24.7[a]	48.0 ± 16.8[a]	40.6 ± 18.3[a]
Boron	32.1 ± 7.32[a]	32.5 ± 6.42[a]	22.4 ± 11.7[b]
Manganese	441 ± 306[a]	440 ± 206[a]	659 ± 413[a]
Iron	2740 ± 821[a]	3620 ± 964[b]	11,000 ± 7730[c]
Copper	8.60 ± 2.02[a]	9.79 ± 1.9[ab]	15.1 ± 7.97[b]
Aluminum	3340 ± 1140[a]	4100 ± 736[a]	4500 ± 1560[a]
Sodium	105 ± 23[a]	111 ± 33.3[a]	122 ± 40.2[a]

found in both marl flats and fens (Fig. 3). *Trichophorum cespitosum* was notable in being restricted mainly to the upper half of axis 2.

For species occurring in five or more quadrats, average maximum leaf height increased significantly with the average midseason LAI in which a species was found (Fig. 4: $r = 0.54$, df = 44, $P < 0.0001$; $r = 0.64$, df = 43, $P < 0.0001$ when the extreme outlier *Typha latifolia* was excluded). The six rare species occurred in microsites with significantly lower LAI than other species (2.13 ± 0.28 vs. 3.36 ± 0.87; one-tailed *t*-test, df = 44, $P < 0.0001$) and had marginally nonsignificant lower leaf heights (25.7 ± 12.1 cm vs. 35.2 ± 18.4 cm; one-tailed *t*-test, df = 44, $P = 0.065$). Leaf height showed an even stronger relationship to July overall vegetation stature in the quadrats in which a given species was present ($r = 0.60$; $r = 0.63$ when *Typha* was excluded). Four common, widely distributed species showed substantial plasticity in leaf height across the study site, with mean leaf height of each increasing with LAI, but with differences between species in leaf height at a given coverage (Fig. 5).

Species richness

Species richness per 0.25 m² declined significantly with midseason LAI (Fig. 6: $y = 15.89 - 1.312$. LAI; $r^2 = 0.21$, df = 189, $P < 0.0001$) and increased with relative slope position ($r = 0.58$, $P < 0.0001$). These results agreed with observations during reconnaissance that sedge meadows were strikingly taller, denser, and less diverse than vegetation on marl flats and fens on peat mounds.

Backward-elimination stepwise regression of species richness on various measures of quadrat environment produced a model that showed species richness decreasing with September quadrat leaf height (H) and peat carbonate content (C), and increasing with slope position (S) and PIC:

$$\text{Species richness} = 12.1 - 0.074[H] + 4.5[S]$$
$$+ 0.12[\text{PIC}] - 0.061[C] \qquad (1)$$

$$(r^2 = 0.43, \text{df} = 189, P < 0.0001)$$

A forward-addition procedure selected only the first two parameters (H and S), which had much greater effects than the others in the backward-elimination model. The backward-elimination model, however, explained a much greater fraction of the variance.

Species richness appeared to show a unimodal relationship to axis 1 scores, increasing slightly from marl flats at the left-hand end of the axis, and then declining through other marl-flat, fen, and sedge-meadow quadrats to the right end of the axis (Fig. 7). However, the initial rise in species richness appears to be driven by a handful of quadrats with very low total coverage.

Phytometer study

Leaf height of *E. perfoliatum*—as well as its proportional biomass allocations to stems and reproductive structures—increased moving
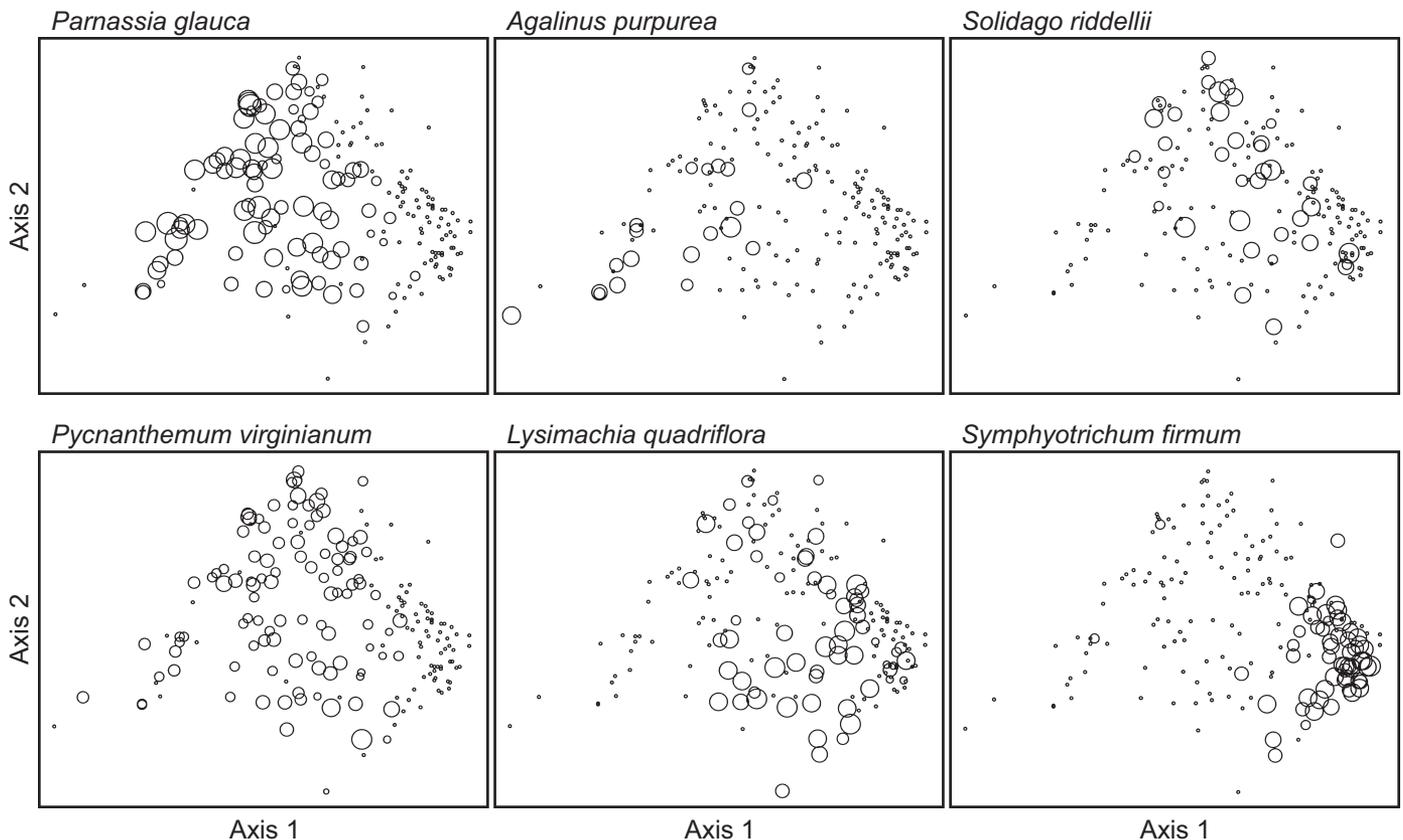
FIGURE 2. Distributions of six representative species of differing maximum leaf heights overlaid on the NMS/BC ordination. The size of the symbol indicates the relative abundance of the species in each quadrat at Clover Valley Scientific Area, Wisconsin, USA. In order of increasing maximum leaf height, the species are *Parnassia glauca*, *Agalinus purpurea*, *Solidago riddellii*, *Pycnanthemum virginianum*, *Lysimachia quadriflora*, and *Symphyotrichum firmum*.
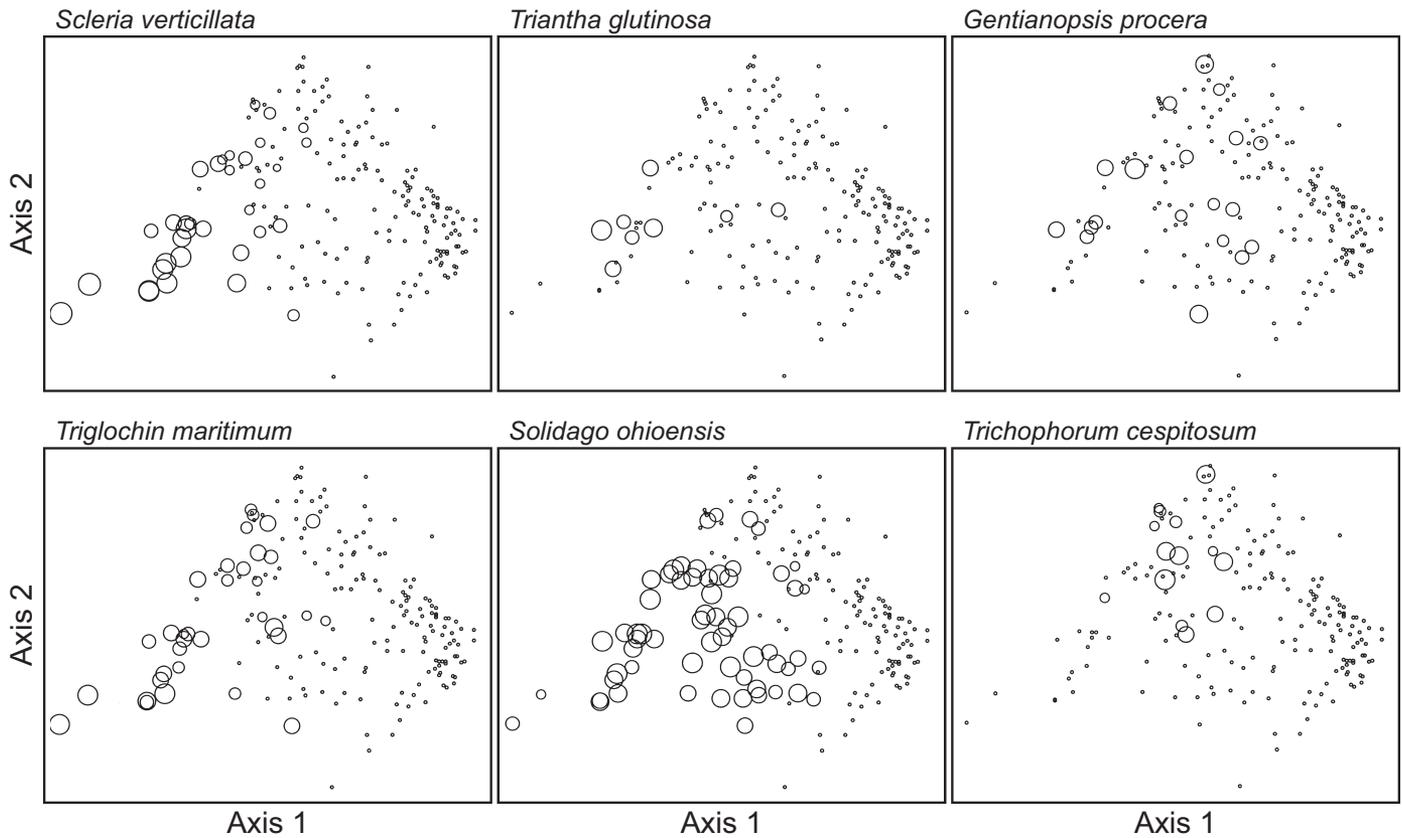
FIGURE 3. Distributions of the six species considered regionally rare in Wisconsin on the NMS/BC ordination, demonstrating their general restriction to short, sparse vegetation in marl-meadow and fen microsites. The size of the symbol indicates the relative abundance of the species in each quadrat at Clover Valley Scientific Area, Wisconsin, USA.

from marl-flat to fen to sedge-meadow quadrats, but most of the differences between vegetation types were not significant (Appendix S4). Leaf N, P, and K content increased moving from marl-flat to fen quadrats, and from fen to sedge-meadow quadrats, with five of nine pairwise differences between vegetation types being significant (Table 4). Leaf Zn content doubled moving from sedge-meadow to marl-flat quadrats and was significantly greater in marl-flat quadrats than in either fen or sedge-meadow quadrats (Table 4). Leaf content of the remaining elements showed no significant differences among vegetation types (Ca, Mg, S, B, Fe, Cu) or small, non-monotonic differences along the marl flat-sedge meadow gradient (Mn, aluminum [Al]).

Remarkably, by far the strongest correlate of leaf height in *E. perfoliatum* (Table 5) was leaf Zn content:

$$\text{Maximum leaf height} = 122.0 - 0.52 \, [\text{Zn}] \qquad (2)$$

$$(r^2 = 0.60, \text{df} = 16, P < 0.001; \text{Fig. 8})$$

The tightness of fit increases to $r^2 = 0.72$ when the most extreme outlier is excluded; leaf N:P, N:K, and K:P ratios each had no significant additional effect on leaf height when entered in a multiple regression with Zn. Leaf height showed significant but weaker increases with leaf P content ($r^2 = 0.39$) and leaf N content ($r^2 = 0.37$). Backward-elimination and forward-addition multiple regression models for leaf height based on leaf elemental concentrations

yielded the same model, with leaf height decreasing with Zn and increasing with Mn:

$$\text{Maximum leaf height} = 86.3 - 0.52 \, [\text{Zn}] + 0.35 \, [\text{Mn}] \qquad (3)$$

$$(r^2 = 0.69, \text{df} = 16, P < 0.0001)$$

Including Mn thus accounted for 9% more of the variance in leaf height than did Zn alone; this represented 22.5% of the residual variance.

Mineral analysis of peat collected from the bases of the 18 specimens of *E. perfoliatum* showed small, nonsignificant declines in Zn concentrations from marl-flat to fen quadrats, and from fen to sedge meadow. By contrast, peat concentrations of P, K, Fe, and Cu increased from marl flat sedge meadows. A more comprehensive analysis of 30 peat samples from across the study site showed the similar trends and some significant differences in peat chemistry among vegetation types (Table 3; see above). A backward-elimination stepwise regression of leaf height based on peat chemistry yielded a model in which height decreased with PIC and increased with substrate concentrations of P, Al, and Na:

$$\text{Maximum leaf height} = 67.6 - 2.32[\text{PIC}] + 0.057[\text{P}]$$
$$+ 0.013[\text{Al}] + 0.342[\text{Na}] \qquad (4)$$

$$(r^2 = 0.76, \text{df} = 16, P < 0.0001)$$
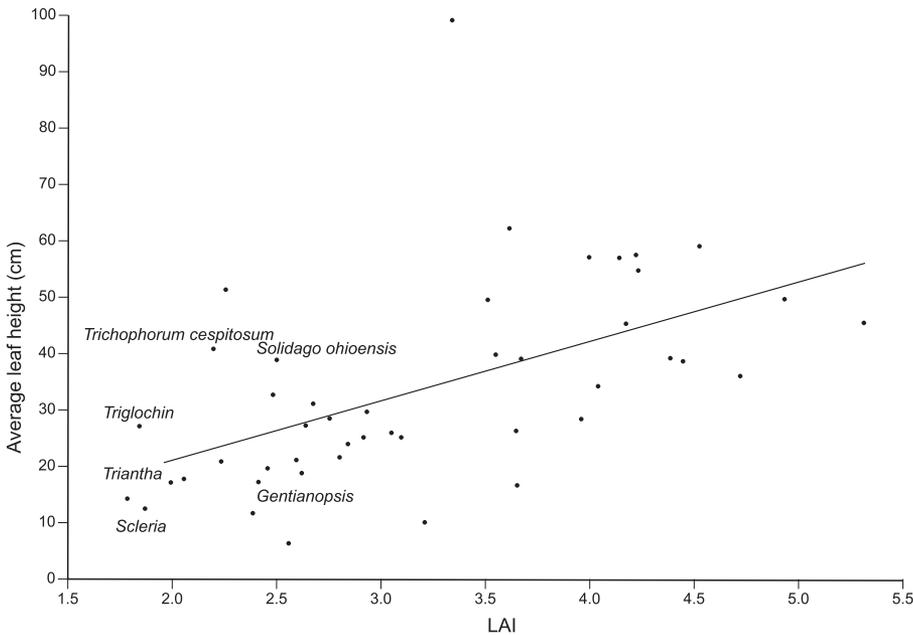
FIGURE 4. Average maximum leaf height vs. the average midseason leaf area index (LAI) of quadrats in which a species is present, for taxa occurring in five or more quadrats at Clover Valley Scientific Area, Wisconsin, USA. Regression line is $y = 10.49x - 1.066$ ($r^2 = 0.41$, $P < 0.0001$ when the extreme outlier *Typha latifolia* is excluded). Regionally rare species are labeled.
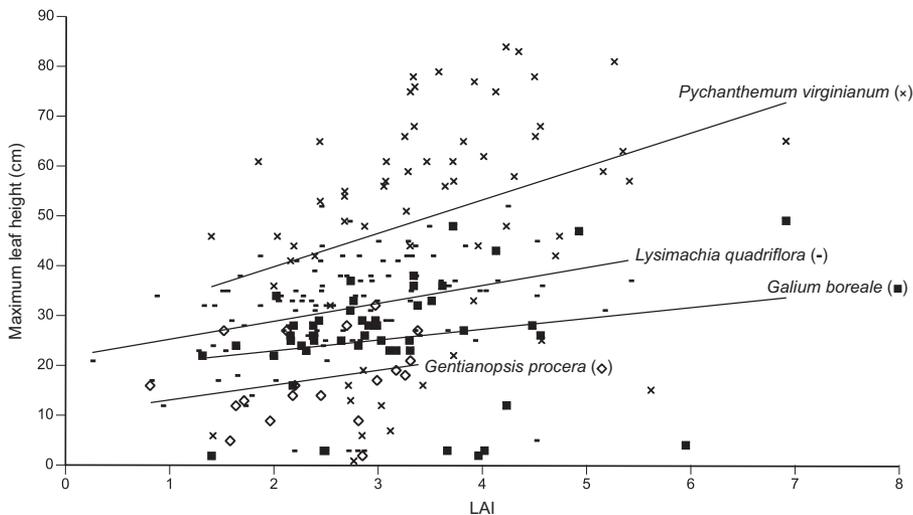
FIGURE 5. Leaf height as a function of midseason leaf area index (LAI) at Clover Valley Scientific Area, Wisconsin, USA, for four widespread species, in order of increasing leaf height: *Gentianopsis procera*, *Galium boreale*, *Lysimachia quadriflora*, and *Pycnanthemum virginianum*. Regression lines relating each species' leaf height to LAI are shown.

The forward-addition procedure yielded only a positive dependence of leaf height on substrate P concentration:

$$\text{Maximum leaf height} = -3.28 + 0.056\,[\text{P}] \qquad (5)$$

$$(r^2 = 0.53, \ df = 16, \ P < 0.0001)$$

Tissue stoichiometry—The leaf N:P ratio for *E. perfoliatum* was 13.7 ± 2.4 in marl flat, 14.3 ± 2.0 in fen, and 7.9 ± 3.7 in sedge meadow (Table 3). Only the sedge-meadow values differed significantly from those in the other two vegetation types. Leaf N:P ratios were in the ambiguous zone (14 < N:P < 16) for N vs. P limitation based on the Koerselman-Meuleman criteria for more than half the *E. perfoliatum* individuals sampled in marl-flat and fen quadrats, while the N:P ratios from five of six individuals in sedge-meadow quadrats clearly supported N limitation. NPK stoichiometry confirmed these results. Based on leaf elemental concentrations for specimens in each vegetation type (Table 3), mean N:K values were 1.9, 2.2, and 1.8 for marl-flat, fen, and sedge-meadow quadrats, respectively, and K:P values were 8.2, 6.5, and 4.5. Based on Olde Venterink et al. (2003), these data clearly identify the sedge-meadow plants as being N limited and classify most marl-flat and fen plants as being P or P+N limited, with the fen plants close to the boundary of N limitation (Fig. 9).

DISCUSSION

Vegetation at the Clover Valley wetland complex appears to reflect a largely one-dimensionsal environmental gradient, in which plant cover and height increase, and substrate carbonate concentration and PIC decrease, moving from marl flats high on the peat mounds downslope toward fens and sedge meadows, reflecting a patchy, roughly one-dimensional spatial gradient of proximity to sources of groundwater discharge (Fig. 1; Tables 2 and 3; Appendices 2 and 3). PIC—associated with the concentration of precipitated carbonates—is highest in the marl flats, nearest the points of groundwater discharge, consistent with the Boyer-Wheeler hypothesis. On several smaller mounds, marl flats are found near the mound summits, while on the larger mounds, they are often found downslope, with shrubby vegetation near the summits. As peat accumulates around points of groundwater discharge, gravitational resistance to vertical flow increases; at some point, lateral seepage downslope of the summit should be favored if paths of lesser resistance through the peat appear, much as side-vents develop downslope of the summits of shield volcanoes. Under those conditions, consistent with the Boyer-Wheeler hypothesis, sparsely covered marl flats should develop on the peat-dome slopes while more productive vegetation takes over the summit as groundwater flow, carbonate precipitation, and P immobilization decline.
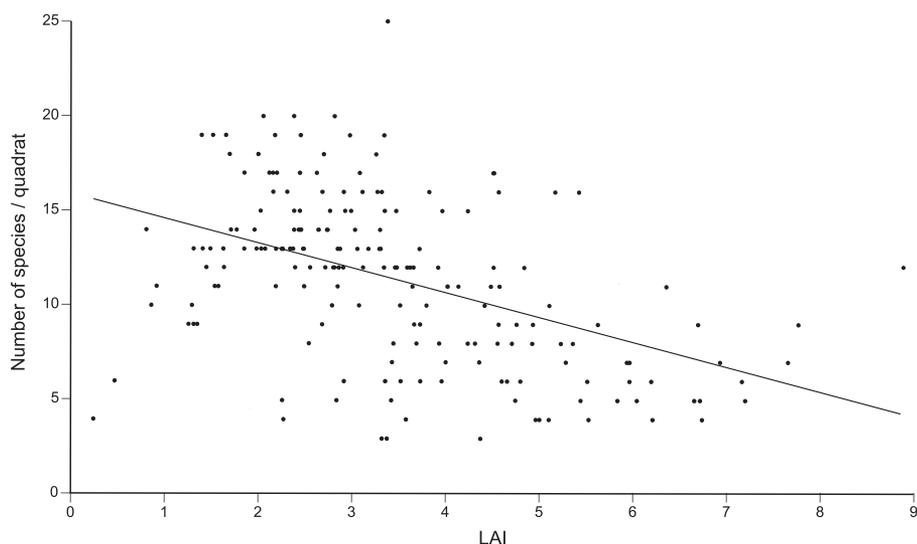
FIGURE 6. Species richness per 0.25 m² as a function of midseason leaf area index (LAI) in this study at Clover Valley Scientific Area, Wisconsin, USA. Fitted line is linear regression, $y = 15.89 - 1.312x$.
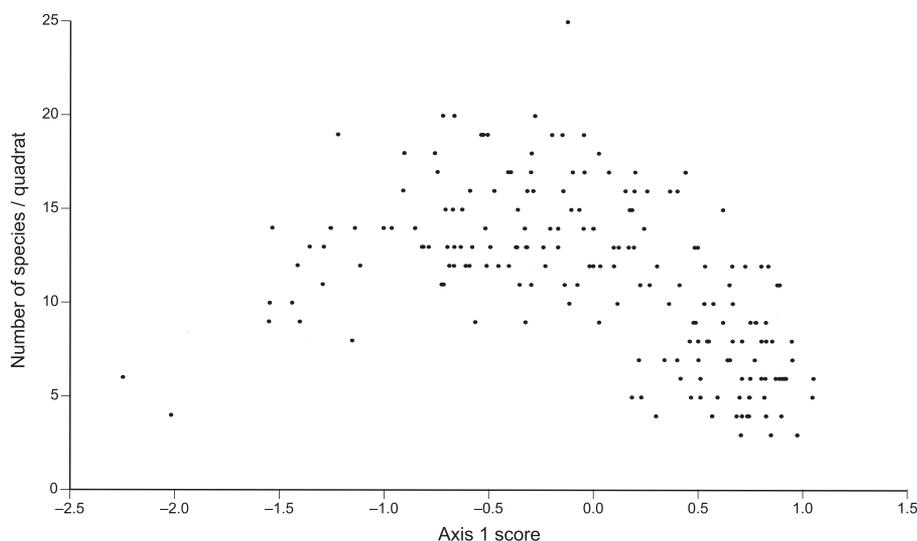
FIGURE 7. Species richness per 0.25 m² as a function of axis 1 score in this study at Clover Valley Scientific Area, Wisconsin, USA.

The 61% increase in total soil P moving from marl flats to sedge meadows is notable. It presumably reflects the decreased dilution by organic matter plus carbonate content toward sedge meadows; the non-carbonate, non-organic soil fraction increased from 14% to 23% moving from marl flats to sedge meadows (see Table 2). The fourfold increase in soil Fe moving from marl flats to sedge meadows parallels an even larger increase in dissolved Fe in soil pore-water along a similar gradient in a fen near Madison, Wisconsin, documented by Kurtz et al. (2007). Those authors attributed that difference to the isolation of the sedge meadows over an impermeable layer from the Fe-poor ground water, combined with increased solubility of Fe under anoxic (low redox potential) conditions and with transpirational concentration of pore-water. Across a survey of 30 European fens, Emsens et al. (2017) found a positive relationship

between soil P and Fe levels, with P limitation apparently being greatest when both soil P and Fe levels were low (3.3 mmol L^{-1} and 15 mmol L^{-1}, respectively).

Species are differentially distributed along the primary vegetation gradient in accord with their differences in leaf height, with taller species being found in areas of greater coverage (Fig. 4); variation in leaf height within four widespread species also increased with plant coverage within individual quadrats (Fig. 5). Both patterns accord the leaf-height model for herbaceous plants advanced by Givnish (1982, 1995): in productive habitats, taller herbs should be favored because the likelihood of being overtopped by neighbors is high, while in less productive areas, the cost of building taller stems confers little photosynthetic advantage and shorter plants are favored. The increase in leaf height with resource supply predicted by Tilman (1988) hinges on precisely this trade-off, although this is not generally recognized. Leach and Givnish (1999) and Wernerehl and Givnish (2015) have also documented significant increases in leaf height with the average coverage in which species occur in Wisconsin savannas and prairies, respectively.

The regionally rare species included in our study occupy significantly more sparsely covered microsites than more common taxa and are substantially shorter in stature (Figs. 3 and 4). This pattern also agrees with the predictions of Givnish (1982, 1995) and with patterns observed in several other communities (e.g., Keddy, 1990; Leach and Givnish, 1996; Farnsworth, 2007; Cornwell and Ackerly, 2010). It seems likely that leaf height per se is an important driver of species distribution in herbs, an important link between plant trait and ecological distribution and abundance. Indeed, Emsens et al. (2018) recently showed that survival of nine endangered *Carex* species transplanted into four Dutch fens increased, as expected, with leaf height and decreased with the density of coverage. Opdekamp et al. (2012) found that cessation of mowing in Polish fens favored taller species and decreased species richness.

Species richness per 0.25 m² decreased with coverage at Clover Valley (Fig. 6), in accord with patterns seen in European fens (e.g., Wheeler and Giller, 1982; Wheeler and Shaw, 1991). This pattern also agrees with theoretical predictions and empirical observations that plant diversity should and do decline with coverage in open herbaceous communities (e.g., Harpole and Tilman, 2007; Hautier et al., 2009; Borer et al., 2014). Hautier et al. (2009) experimentally demonstrated that this decline in species richness at greater coverage is a direct consequence of shading and exclusion of shorter species, by showing that increasing light availability at ground level reverses it. Grime (1979) proposed that plant diversity should instead rise, then fall along a gradient of increasing productivity,

TABLE 4. Concentrations (mean ± SD) of various nutrients and NPK stoichiometry in the leaf tissue of six individuals of *Eupatorium perfoliatum* in each of the three vegetation zones at Clover Valley Scientific Area, Wisconsin, USA. Significant differences (pairwise two-tailed *t*-tests, df = 10, *P* < 0.05) are indicated by different superscripts. Aluminum, iron, and magnesium show the least proportional variation across zones (~12%), while phosphorus (175%) and zinc (96%) shows the greatest variation.

Nutrient	Marl flat	Fen	Sedge meadow
Nitrogen (%)	1.10 ± 0.10[a]	1.53 ± 0.18[b]	1.56 ± 0.32[b]
Phosphorus (%)	0.08 ± 0.02[a]	0.11 ± 0.01[a]	0.22 ± 0.06[b]
Potassium (%)	0.64 ± 0.17[a]	0.71 ± 0.17[ab]	0.89 ± 0.19[b]
Calcium (%)	2.55 ± 0.33[a]	2.50 ± 0.27[a]	2.95 ± 0.48[a]
Magnesium (%)	0.93 ± 0.18[a]	0.90 ± 0.20[a]	0.82 ± 0.12[a]
Sulfur (%)	0.62 ± 0.21[a]	0.67 ± 0.22[a]	0.79 ± 0.12[a]
Zinc (ppm)	120 ± 25[a]	82.2 ± 29[b]	61.1 ± 20[b]
Boron (ppm)	28.9 ± 9.8[a]	27.0 ± 8.7[a]	32.9 ± 11[a]
Manganese (ppm)	93.8 ± 34[ab]	95.4 ± 9.0[a]	117 ± 13[b]
Iron (ppm)	114 ± 16[a]	105 ± 4.3[a]	118 ± 19[a]
Copper (ppm)	13.3 ± 7.0[a]	17.7 ± 4.4[a]	21.5 ± 6.1[a]
Aluminum (ppm)	103 ± 12.6[ab]	92.9 ± 3.9[a]	104 ± 9.8[b]
Nitrogen : phosphorus	13.7 ± 2.4[a]	14.3 ± 2.0[a]	7.9 ± 3.7[b]
Nitrogen : potassium	1.86 ± 0.70[a]	2.23 ± 0.45[a]	1.80 ± 0.42[a]
Potassium : phosphorus	8.21 ± 3.2[ab]	6.52 ± 0.90[a]	4.48 ± 1.87[b]

TABLE 5. Correlations (*r*) of *Eupatorium perfoliatum* maximum leaf height with chemical composition of leaf tissue and the peat substrate in which the plants were rooted at Clover Valley Scientific Area, Wisconsin, USA. Significance of correlations indicated by * (0.05), ** (0.01), and *** (0.001).

Nutrient	Leaf tissue	Substrate
Nitrogen (%)	0.612**	
Phosphorus (%)	0.628**	0.726***
Potassium (%)	0.347	−0.098
Calcium (%)	0.302	−0.234
Magnesium (%)	−0.263	−0.468*
Sulfur (%)	0.028	−0.256
Zinc (ppm)	−0.777***	−0.253
Boron (ppm)	0.198	−0.679**
Manganese (ppm)	0.362	−0.100
Iron (ppm)	0.003	0.415
Copper (ppm)	0.383	0.478*
Aluminum (ppm)	−0.117	0.154
Sodium (ppm)		0.307
Organic matter (%)		0.159
Carbonate (%)		−0.191
Phosphorus immobilization capacity		−0.014

FIGURE 8. Leaf height of *Eupatorium perfoliatum* as a function of leaf Zn concentration in this study at Clover Valley Scientific Area, Wisconsin, USA (fitted line is linear regression).
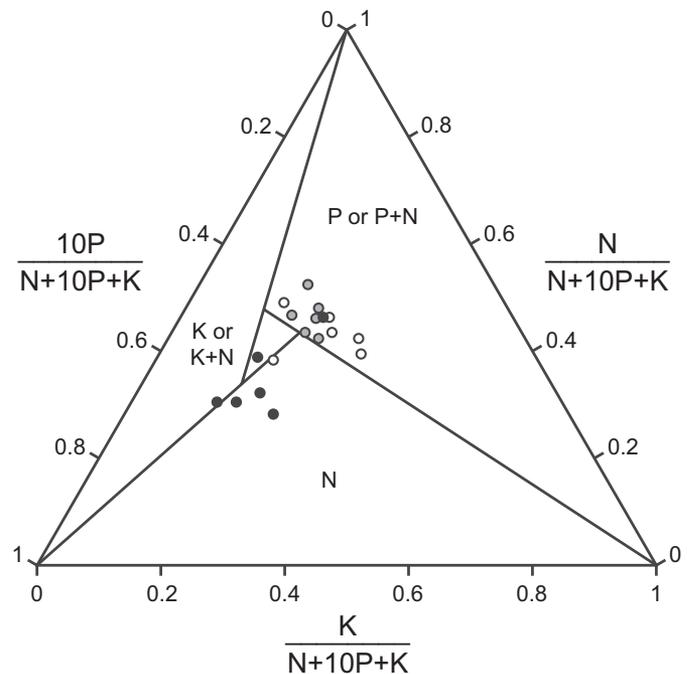
FIGURE 9. Ternary plot of NPK stoichiometry of *Eupatorium perfoliatum* from marl-flat, fen, and sedge-meadow quadrats (unfilled, gray-filled, and black-filled circles, respectively) at Clover Valley Scientific Area, Wisconsin, USA. Lines represent boundaries between conditions implying N limitation, P or P+N limitation, and K or K+N limitation established by Olde Venterink et al. (2003) based on outcomes of experimental fertilizations in European wetlands; the smallest triangle represents a zone in which the particular form of nutrient limitation cannot be inferred from leaf stoichiometry.

based on intense competitive exclusion under productive conditions and by limitation of species pool size by unproductive conditions. This proposal has spawned much controversy, with Adler et al. (2011) claiming there was no global regularity in the plant diversity–productivity relationship, and Fraser et al. (2015a, b) arguing instead that, if extremely productive and unproductive sites are included, grasslands show the hypothesized unimodal relationship. Our data support a simple decline of species richness with coverage (Fig. 6), but species richness also rises, then falls moving from marl flats to fens to sedge meadows along ordination axis 1 (Fig. 7). The apparent drop in species richness at the left-hand extreme of axis 1, however, is driven by a handful of quadrats with very sparse coverage that, perforce, have few species present.

Charging of water percolating
through limestone/dolomite
with carbon dioxide
and carbonates

↓

Degassing at springheads

↓

Co-precipitation of P with calcium carbonate

↓

P sequestration

Reduction in N fixation

Reduced complexing of P with Zn

N limitation　　　P limitation　　　Zn toxicity

Reduced plant density, stature

↓

Favorable conditions for short, rare species

FIGURE 10. Proposed schema of the hydrogeochemical processes maintaining sparse vegetation—rich in short-statured, regionally rare species—near calcareous springheads at Clover Valley Scientific Area, Wisconsin, USA.

Our findings regarding the limitations of plant coverage in calcareous spring-fed wetlands were consistent with only certain aspects of the Boyer-Wheeler hypothesis. As predicted by the latter, carbonate content and P immobilization concentration of the substrate increased near the groundwater sources, but N:P ratios and NPK stoichiometry supported a shift toward P or P+N co-limitation near groundwater sources, not P limitation (Table 4; Fig. 9). Furthermore, there is strong circumstantial evidence that Zn toxicity may help restrict coverage near springheads, based on the doubling of leaf Zn concentrations in *E. perfoliatum* growing in marl flats vs. sedge meadows, and the strong decline in *E. perfoliatum* height with leaf Zn levels (Fig. 8). A limitation of our data is that we measured leaf elemental concentrations for only a single (albeit widespread) species; taxa may differ in their response to fertilization by different elements (e.g., see Øien, 2004). Stoichiometric measurements on a broader sampling of species across several fen/sedge-meadow gradients would provide important tests of the generality of our conclusions regarding NPK limitations and Zn toxicity along springhead–sedge meadow gradients.

We therefore propose two modifications of the Boyer-Wheeler hypothesis (Fig. 10). First, while P availability near springheads should—in accord with that hypothesis—be reduced by P co-precipitating with calcium carbonate as groundwater degasses, the resulting P shortage should also reduce N availability and result in co-limitation by P and N. Availability to plants of reactive N (nitrate or ammonium) should reflect the importance of N fixation by soil microbes vis-à-vis microbial denitrification, and the high demand for P by fixers given the large energy demands (and thus, ATP requirements) of N fixation (see Pate, 1986; Vitousek and Field, 1999). In European mires, soil rates of N fixation reach a peak under moderately acid conditions (pH 5.0–6.5) and decline under more acid or more alkaline conditions (Malmer, 1986). We believe that this pattern reflects a peak in P availability at intermediate pH, caused by P complexing with Fe at low pH as Fe becomes more mobile, especially under anoxic conditions (Manning et al., 1991; Rothe et al., 2014; Emsens et al., 2017), and by P complexing with $CaCO_3$ at high pH (Boyer and Wheeler, 1989; Richardson and Vaithiyanathan, 2009; Givnish, 2002: appendix). In addition, N

fixation is likely to be low, while denitrification remains high in more anoxic soils under sedge meadows with standing water, remote from springheads, further lowering reactive N availability. Finally, the greater combined accumulation of carbonates and organic matter near springheads (Table 3) should dilute the supplies of soil P even more than Boyer and Wheeler (1989) envisioned. We propose that the Boyer-Wheeler hypothesis should thus be extended to incorporate the cascading effects of calcareous springheads on calcium carbonate precipitation, P co-precipitation and availability, N fixation, denitrification, and P and N co-limitation (Fig. 10). Indeed, Øien et al. (2018) recently found strong evidence for co-limitation of fen production by P and N in a 15 yr study involving factorial application of N, P, and K.

Second, we propose that P co-precipitation should have another cascading effect, increasing the likelihood of Zn toxicity. It is highly intriguing that leaf Zn concentration was the strongest single predictor of *E. perfoliatum* stature. The average leaf Zn concentration of 120 ppm in *E. perfoliatum* in marl flats approximates the levels considered excessive in crops (>75 ppm for soybeans, >100 ppm for alfalfa and oats, >150 ppm for maize; Schulte, 1992). This is especially surprising because Zn solubility decreases with increasing pH, and because there were no significant differences among vegetation types in substrate Zn concentration (Table 3). So leaf Zn concentrations appear to approach toxic levels near calcareous springheads at our site, even though substrate Zn concentrations there did not differ significantly from those in the surrounding sedge meadows. In upland soils, P and Zn can complex and be sorbed insolubly onto soil particles at circumneutral pH (Agbenin, 1998), leading to possible deficiencies of absorption in either element caused by applications of the other (e.g., Xie et al., 1989; Schulte, 1992; Jones, 1998; Lu et al., 1998; Bogdanovic et al., 1999). We therefore propose that P sequestration in carbonates near springheads may free up Zn availability and lead to high levels of plant absorption, resulting in mild Zn toxicity and reduced plant production and coverage (Fig. 10).

A full understanding of the hydrogeochemical processes that create patches of sparsely covered, highly diverse microsites within calcareous wetlands is critical to developing strategies to conserve the overall plant and animal diversity of fens. Outright destruction, drainage, and heavy mechanical damage to soils and vegetation have already claimed large numbers of fens throughout the U.S. Midwest (Bedford and Godwin, 2003; Nekola, 2004) and Europe (Rydin and Jeglum, 2013). Our model suggests that the greatest remaining human threats to these biodiversity hotspots would involve disruption of local hydrological regimes and associated geochemical processes that help maintain an ecologically diverse range of microsites. Drainage, well construction, or shifts in precipitation/evaporation ratios caused by anthropogenic climate change that have little or no impact on local water levels in fens, but that substantially decrease the rate of *flow* from springheads, could easily result in local loss of rare, short-statured species by decreasing N and P limitation, by increasing Zn toxicity, by reducing sulfide release (Simkin et al., 2013), or by allowing soils near springheads to warm and release more nutrients to plant roots (Horsák et al., 2018). To the extent that fens are co-limited by both P and N, increased atmospheric N deposition resulting from human activities may also have adverse impacts on fen biodiversity, just as it has on rare plants in European chalk grasslands (e.g., see Bobbink et al., 1998). Eutrophication through release of phosphates or nitrates into aquifers (Wassen et al., 1988; Wheeler, 1988; Koerselman et al., 1990), or through repeated desiccation via well withdrawal and rewetting, is also a key threat (van Diggelen et al., 2006; Emsens et al., 2018).

ACKNOWLEDGEMENTS

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Map of study transects.

APPENDIX S2. Correlation matrix of environmental parameters.

APPENDIX S3. Environmental parameters for marl-flat, fen, and sedge-meadow quadrats.

APPENDIX S4. Height, mass, and biomass allocation fractions for *Eupatorium perfoliatum* in each vegetation zone.

LITERATURE CITED

Adler, P. B., E. W. Seabloom, E. T. Borer, H. Hillebrand, Y. Hautier, A. Hector, W. S. Harpole, et al. 2011. Productivity is a poor predictor of plant species richness. *Science* 333: 1750–1753.

Agbenin, J. O. 1998. Phosphate-induced zinc retention in a tropical semi-arid soil. *European Journal of Soil Science* 49: 693–700.

Amon, J. P., C. A. Thompson, Q. J. Carpenter, and J. Miner. 2002. Temperate zone fens of the glaciated Midwest. *Wetlands* 22: 301–317.

Bedford, B. L., and K. S. Godwin. 2003. Fens of the United States: distribution, characteristics, and scientific connection versus legal isolation. *Wetlands* 23: 608–629.

Bedford, B. L., M. R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversioty of temperate North American wetlands. *Ecology* 80: 2151–2169.

Bobbink, R., M. Hornung, and J. G. M. Roelofs. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology* 86: 717–738.

Boeye, D., B. Verhagen, V. Van Haesebroeck, and R. F. Verheyen. 1997. Nutrient limitation in species-rich lowland fens. *Journal of Vegetation Science* 8: 415–424.

Bogdanovic, D., M. Ubavic, and M. Cuvardic. 1999. Effect of phosphorus fertilization on Zn and Cd contents in soil and corn plants. *Nutrient Cycling in Agroecosystems* 54: 49–56.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. Stanley Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, et al. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508: 517–520.

Bowles, M. L., P. D. Kelsey, and J. L. McBride. 2005. Relationships among environmental factors, vegetation zones, and species richness in a North America calcareous prairie fen. *Wetlands* 25: 685–696.

Boyer, M. L. H., and B. D. Wheeler. 1989. Vegetation patterns in spring-fed calcareous fens: calcite precipitation and constraints on fertility. *Journal of Ecology* 77: 597–609.

Carpenter, Q. J. 1995. Toward a new definition of calcareous fen for Wisconsin (USA). Ph.D. thesis, University of Wisconsin-Madison.

Ciolkosz, E. J. 1965. Peat mounds of southeastern Wisconsin. *Soil Science Survey* 1: 15–17.

Cornwell, W. K., and D. D. Ackerly. 2010. A link between plant traits and abundance: evidence from coastal California woody plants. *Journal of Ecology* 98: 814–821.

Curtis, J. T. 1946. Use of mowing in management of white ladyslipper. *Journal of Wildlife Management* 10: 303–308.

Curtis, J. T. 1959. The vegetation of Wisconsin. University of Wisconsin Press, Madison, WI.

Cusell, C., A. Kooijman, and L. P. M. Lamers. 2014. Nitrogen or phosphorus limitation in rich fens? Edaphic differences explain contrasting results in vegetation development after fertilization. *Plant Soil* 384: 153–168.

Dean, W. E. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. *Journal of Sedimentary Petrology* 44: 242–248.

Duval, T. P., and J. M. Waddington. 2018. Effect of hydrogeomorphic setting on calcareous fen hydrology. *Hydrological Processes* 32: 1695–1708.

Emsens, W. J., C. J. S. Aggenbach, A. J. P. Smolders, D. Zak, and R. van Diggelen. 2017. Restoration of endangered fen communities: the ambiguity of iron-phosphorus binding and phosphorus limitation. *Journal of Applied Ecology* 54: 1755–1764.

Emsens, W. J., C. J. S. Aggenbach, H. Rydin, A. J. P. Smolders, and R. van Diggelen. 2018. Competition for light as a bottleneck for endangered fen species: an introduction experiment. *Biological Conservation* 220: 76–83.

Farnsworth, E. J. 2007. Plant life history traits of rare versus frequent plant taxa of sandplains: implications for research and management trials. *Biological Conservation* 136: 44–52.

Fojt, W. J. 1994. Dehydration and the threat to East Anglian fens, England. *Biological Conservation* 69: 163–175.

Fojt, W., and M. Harding. 1995. Thirty years of change in the vegetation communities of three valley mires in Suffolk, England. *Journal of Applied Ecology* 32: 561–577.

Fraser, L. H., J. Pither, A. Jentsch, M. Sternberg, M. Zobel, D. Askarizadeh, S. Bartha, et al. 2015a. Worldwide evidence of a unimodal relationship between productivity and plant species richness. *Science* 349: 302–305.

Fraser, L. H., M. Pärtel, J. Pither, A. Jentsch, M. Sternberg, and M. Zobel. 2015b. Response to Comment on "Worldwide evidence of a unimodal relationship between productivity and plant species richness". *Science* 350: 1177c.

Givnish, T. J. 1982. On the adaptive significance of leaf height in forest herbs. *American Naturalist* 120: 353–381.

Givnish, T. J. 1995. Plant stems: biomechanical adaptation for energy capture and influence on species distributions. *In* B. L. Gartnet [ed.], Plant stems: physiology and functional morphology, 3–49. Academic Press, New York.

Givnish, T. J. 2002. On the adaptive significance of evergreen vs. deciduous leaves: solving the triple paradox. *Silva Fennica* 36: 703–743.

Grime, J. P. 1979. Plant strategies and vegetation processes. Wiley, London.

Harding, M. 1993. Redgrave and Lopham Fens, East Anglia, England: a case study of change in flora and fauna due to groundwater abstraction. *Biological Conservation* 66: 35–45.

Harpole, W. S., and D. Tilman. 2007. Grassland species loss resulting from reduced niche dimension. *Nature* 446: 791–793.

Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* 324: 636–638.

Horsákova, V., M. Hájek, P. Háková, D. Dité, and M. Horsák. 2018. Principal factors controlling the species richness of European fens differ between habitat specialists and matrix-derived species. *Diversity and Distributions* 24: 742–754.

Jacobson, G. L., Jr., H. Almquist-Jacobson, and J. C. Winne. 1991. Conservation of rare plant habitat: insights from the recent history of vegetation and fire at Crystal Fen, northern Maine, USA. *Biological Conservation* 57: 287–314.

Jímenez-Alfaro, B., E. Fernando-Pascual, T. E. D. Gonzalez, A. Perez-Haase, and J. M. Ninot. 2012. Diversity of rich fen vegetation and related plant specialists in mountain refugia of the Iberian Peninsula. *Folia Geobotanica* 47: 403–419.

Jímenez-Alfaro, B., S. Suarez-Seoane, M. Chytry, S. M. Hennekens, W. Willner, M. Hajek, E. Agrillo, et al. 2018. Modeling the distribution and compositional variation of plant communities at the continental scale. *Diversity and Distributions* 24: 978–990.

Jones, J. B. 1998. Phosphorus toxicity in tomato plants: when and how does it occur? *Communications in Soil Science and Plant Analysis* 29: 1779–1784.

Keddy, P. A. 1990. Competitive hierarchies and centrifugal organization in plant communities. *In* J. B. Grace and D. Tilman [eds.], Perspectives on plant competition, 265–290. Academic Press, New York.

Koerselman, W., and A. F. M. Meuleman. 1996. The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33: 1441–1450.

Koerselman, W., S. A. Bakker, and M. Blom. 1990. Nitrogen, phosphorus and potassium budgets for two small fens surrounded by heavily fertilized pastures. *Journal of Ecology* 78: 428–442.

Kratz, T. K., M. J. Winkler, and C. B. DeWitt. 1981. Hydrology and chronology of a peat mound in Dane County, southern Wisconsin. *Wisconsin Academy of Sciences and Letters* 69: 37–45.

Kurtz, A. M., J. M. Bahr, Q. J. Carpenter, and R. J. Hunt. 2007. The importance of subsurface geology for water source and vegetation communities in Cherokee Mark, Wisconsin. *Wetlands* 27: 189–202.

Leach, M. K., and T. J. Givnish. 1996. Ecological determinants of species loss in remnant prairies. *Science* 273: 1555–1558.

Leach, M. K., and T. J. Givnish. 1999. Gradients in the composition, structure, and diversity of remnant oak savannas. *Ecological Monographs* 69: 353–374.

Lu, Z. G., H. S. Grewal, and R. D. Graham. 1998. Dry matter production and uptake of zinc and phosphorus in two oilseed rape genotypes under differential rates of zinc and phosphorus supply. *Journal of Plant Nutrition* 21: 25–38.

Malmer, N. 1986. Vegetational gradients in relation to environmental conditions in northwestern European mires. *Canadian Journal of Botany* 64: 375–383.

Manning, P. G., T. P. Murphy, and E. E. Prepas. 1991. Intensive formation of vivianite in the bottom sediments of mesotrophic Narrow Lake, Alberta. *Canadian Mineralogist* 29: 77–85.

McCune, B., and M. J. Mefford. 1997. *PC-ORD: multivariate analysis of ecological data* (version 3.15). MjM Software Design, Glenenden Beach, Oregon, USA.

Menichino, N. M., N. Fenner, A. S. Pullin, P. S. Jones, J. Guest, and L. Jones. 2016. Contrasting response to mowing in two abandoned rich fen plant communities. *Ecological Engineering* 86: 210–222.

Miner, J. J., and D. B. Ketterling. 2003. Dynamics of peat accumulation and marl flat formation in a calcareous fen, Midwestern United States. *Wetlands* 23: 950–960.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytical Chemistry Acta* 27: 31–36.

Nekola, J. C. 2004. Vascular plant compositional gradients within and between Iowa fens. *Journal of Vegetation Science* 15: 771–780.

Nieminen, M., and M. Jarva. 1996. Phosphorus adsorption by peat from drained mires in southern Finland. *Scandinavian Journal of Forestry Research* 11: 321–326.

Øien, D. I. 2004. Nutrient limitation in boreal rich-fen vegetation: a fertilization experiment. *Applied Vegetation Science* 7: 119–132.

Øien, D. I., B. Pedersen, L. Kozub, K. Goldstein, and M. Wilk. 2018. Long-term effects of nutrient enrichment controlling species and functional composition in a boreal rich fen. *Journal of Vegetation Science* 29: 907–920.

Olde Venterink, H., M. J. Wassen, A. W. M. Verkroost, and P. C. de Ruiter. 2003. Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. *Ecology* 84: 2191–2199.

Opdekamp, W., O. Beauchard, H. Backx, F. Franken, T. J. S. Cox, R. van Diggelen, and P. Meire. 2012. Effects of mowing cessation and hydrology on plant trait distribution in natural fen meadows. *Acta Oecologica* 39: 117–127.

Pate, J. S. 1986. The economy of symbiotic nitrogen fixation. *In* T. J. Givnish [ed.], On the economy of plant form and function, 299–325. Cambridge University Press, Cambridge.

Raney, P. A., and D. J. Leopold. 2018. Fantastic wetlands and where to find them: modeling rich fen distribution in New York State with Maxent. *Wetlands* 38: 81–93.

Richardson, C. J., and P. Vaithiyanathan. 2009. Biogeochemical dynamics II: cycling and storage of phosphorus in wetlands. *In* E. Maltby and T. Barker [eds.], The Wetlands Handbook, 2 Volume Set, 228–248. Wiley-Blackwell, Chichester, UK.

Rothe, M., T. Frederichs, M. Eder, A. Kleeberg, and M. Hupfer. 2014. Evidence for vivianite formation and its contribution to long-term phosphorus retention in a recent lake sediment: a novel analytical approach. *Biogeosciences* 11: 5169–5180.

Rozbrojová, Z., and M. Hájek. 2008. Changes in nutrient limitation of spring fen vegetation along environmental gradients in the West Carpathians. *Journal of Vegetation Science* 19: 613–620.

Rydin, H., and J. K. Jeglum. 2013. The biology of peatlands. (2nd ed.). Oxford University Press, Oxford, United Kingdom.

Schulte, E. E. 1992. Soil and applied zinc. University of Wisconsin-Extension, Madison, WI.

Seer, F. K., and J. Schrautzer. 2014. Status, future prospects, and management recommendations for alkaline fens in an agricultural landscape: a comprehensive survey. *Journal for Nature Conservation* 22: 358–368.

Simkin, S. M., B. L. Bedford, and K. C. Weathers. 2013. Phytotoxic sulfide more important than nutrients for plants within a groundwater-fed wetland. *Ecosystems* 16: 1118–1129.

Sjörs, H. 1950. On the relation between vegetation and electrolytes in north Swedish mire waters. *Oikos* 2: 241–258.

Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton.

van Diggelen, R., W. J. Molenaar, and A. M. Kooijman. 1996. Vegetation succession in a floating mire in relation to management and hydrology. *Journal of Vegetation Science* 7: 809–820.

van Diggelen, R., B. Middleton, J. Bakker, A. Grootjans, and M. Wassen. 2006. Fens and floodplains of the temperate zone: present status, threats, conservation and restoration. *Applied Vegetation Science* 9: 157–162.

Verhoeven, J. T. A., W. Koerselman, and B. Beltman. 1988. The vegetation of fens in relation to their hydrology and nutrient dynamics: A case study. *In* J. J. Symoens [ed.], Vegetation of inland waters, 249–282. Kluwer Academic Publishers, Dordrecht.

Vitousek, P. M., and C. B. Field. 1999. Ecosystem constraints to symbiotic nitrogen fixers: a simple model and its implications. *Biogeochemistry* 46: 179–202.

Vojtech, E., L. A. Turnbull, and A. Hector. 2007. Differences in light interception in grass monocultures predict short-term competitive outcomes under productive conditions. *PLoS One* 6: e499.

Wassen, M. J., A. Barendregt, M. C. Bootsma, and P. P. Schot. 1988. Groundwater chemistry and vegetation of gradients from rich fen to poor fen in the Naardermeer (the Netherlands). *Vegetatio* 79: 117–132.

Wernerehl, R. W., and T. J. Givnish. 2015. Relative roles of soil moisture, nutrient supply, depth, and mechanical impedance in determining composition and structure of Wisconsin prairies. *PLoS One* 10: e0137963.

Wheeler, B. D. 1988. Species richness, species rarity and conservations evaluation of rich-fen vegetation in lowland England and Wales. *Journal of Applied Ecology* 25: 331–353.

Wheeler, B. D., and K. E. Giller. 1982. Species richness of herbaceous fen vegetation in Broadland, Norfolk in relation to the quantity of above-ground plant material. *Journal of Ecology* 70: 179–200.

Wheeler, B. D., and S. C. Shaw. 1991. Above-ground crop mass and species richness of the principal types of herbaceous rich-fen vegetation of lowland England and Wales. *Journal of Ecology* 79: 285–301.

Xie, R. J., and A. F. MacKenzie. 1989. Effects of sorbed orthophosphate on zinc status in three soils of eastern Canada. *Journal of Soil Science* 40: 49–58.