

MOSSES INFLUENCE VASCULAR PLANTS IN RICH FENS THROUGH
EFFECTS ON THE BIOGEOCHEMISTRY OF SHALLOW SOILS

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Surprisingly little is known about the effects of non-*Sphagnum* mosses on soil processes or associated vascular plants, despite known strong effects of *Sphagnum* on the biogeochemistry of acidic peatlands. Mineral-rich fens, which are groundwater-fed peatlands where phosphorus often limits plant growth, frequently have a dominant layer of "brown" mosses covering the peat surface and an unusually high diversity of forb species rooted in shallow soils. I hypothesized that rich fen mosses would have biogeochemical effects that in turn would facilitate forb species both below- and aboveground, thereby influencing plant species diversity overall.

To investigate the effects of rich fen mosses on biogeochemical processes, I performed a moss removal experiment at three New York fens. Where mosses occurred, redox potential was greater, reduced iron concentrations were lower, and phosphorus uptake by plants was higher than where mosses were removed. More oxidized soil conditions underlying mosses may have stimulated (1) greater phosphate supply through increased microbial mineralization; (2) greater phosphorus demand via microbial uptake, plant uptake, and iron-phosphorus reactions; and (3) greater phosphorus acquisition through improved plant vigor and mycorrhizal colonization—thus improving forb phosphorus uptake in a phosphorus-limited environment.

Below- and aboveground responses of vascular plants to mosses were examined in the moss removal experiment and in a survey of moss-covered and naturally moss-free areas. Total root biomass and root length density from shallow soil core sections were greater with mosses present than naturally absent. For two forb

species tested experimentally, root length, surface area, and branching were 1.5-2 times greater where mosses occurred. Forb seedling survival, numbers of individuals, percent cover, and species density all were greater where mosses occurred; in contrast, graminoids did not respond. Patterns of root morphology and species establishment were consistent with demonstrated biogeochemical effects of mosses on redox potential and phosphorus cycling in shallow soils.

This work emphasizes the role of positive plant species interactions in rich fens, suggesting that mosses may help diverse, shallowly rooted forb species to succeed under saturated and phosphorus-limited conditions. The presence of mosses thus may have key implications for the plant species composition and diversity of these unique wetlands.

BIOGRAPHICAL SKETCH

Katherine Crowley studied botany and zoology at Michigan State University and received a Bachelor of Science degree in 1992. In 1995 she earned a Master of Science degree in ecology working with Edward Cushing and Kenneth Cole at the University of Minnesota, where she investigated patterns of temporal and spatial change in the vegetation of Voyageurs National Park. Following her undergraduate studies and Masters work, Katherine was employed in the private, non-profit, and public sectors, initially as a regional botany specialist for the Midwest Regional Office of The Nature Conservancy. She next worked on wetland regulatory projects as a plant ecologist for Huffman & Associates, Inc. and Huffman & Carpenter, Inc. in Reno, Nevada. Subsequently, Katherine again worked for The Nature Conservancy through the New Hampshire Natural Heritage Bureau, where she implemented inventory and research projects related to conservation of New Hampshire's natural communities and rare plant species. During this time she also taught an introductory environmental science course at St. Anselm College, a rewarding experience that inspired her to pursue a doctoral program in wetland plant ecology and biogeochemistry at Cornell University. Katherine will shortly begin a postdoctoral associate position with Gary Lovett through the Cary Institute of Ecosystem Studies, where she will analyze biogeochemical trends across the Northeast. Katherine currently lives on a farm in Liberty, Maine, where she is helping to raise a large garden and five chickens.

I dedicate this work to my parents, Judi and Fred, who inspired me to believe that I could accomplish anything; to my brother Glenn, who believed in me even when we were children, and still does; and to my cousin Chris, one of the best people I know.

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CHAPTER 1

MOSSES INFLUENCE REDOX CONDITIONS AND PHOSPHORUS CYCLING IN SHALLOW SOILS OF RICH FENS IN CENTRAL NEW YORK

ABSTRACT

Surprisingly little is known about the effects of non-*Sphagnum* mosses on soil processes, despite known strong effects of *Sphagnum* species on the biogeochemistry of acidic peatland environments. In mineral-rich fens, which are groundwater-fed peatlands where phosphorus availability often limits plant growth, the biogeochemical effects of the "brown" mosses may be important because these systems have a high diversity of plant species that are shallowly rooted and therefore in the zone of greatest moss influence. To investigate the role of mosses in rich fen phosphorus cycling, I established a moss removal experiment in three New York fens. In the presence of mosses, redox potential was greater (more oxidized), concentrations of reduced iron were lower, and phosphate supply to anion exchange membrane (AEM) resins was up to 60% lower than where mosses had been removed. Furthermore, soil phosphatase activity, root phosphatase activity, and root colonization by arbuscular mycorrhizal fungi (AMF) all were correspondingly higher underlying mosses, consistent with typical responses to lower phosphorus availability. However, microbial biomass, microbial phosphorus, and forb tissue phosphorus concentrations were higher in the presence of mosses, consistent with greater phosphorus availability and non-competitive phosphorus uptake. More oxidized conditions underlying mosses simultaneously may have stimulated (1) greater phosphate supply through increased microbial mineralization, possibly promoted also by greater labile carbon supply; (2) higher phosphorus demand via microbial uptake, plant uptake, and geochemical iron-phosphorus reactions; and (3) greater phosphorus acquisition through improved plant

vigor and root colonization by AMF. Moss effects on redox conditions in shallow soils thus may improve forb phosphorus uptake in a phosphorus-limited environment, which has important implications for the species composition and diversity of these unique systems.

INTRODUCTION

Individual organisms or groups of organisms may modify the physical and chemical characteristics of an ecosystem in ways that have significant consequences for biogeochemical processes and habitat conditions. Gutierrez and Jones (2006) suggest that such physical "ecosystem engineers" (Jones et al. 1994) modify their environment by transferring either materials or heat, which in turn may influence nutrient availability, carbon availability to microbes, or physical factors such as pH, temperature, or soil moisture. The effects of ecosystem engineers on the physical and chemical environments of many systems, with subsequent effects on biogeochemical processes and the abundance and composition of other organisms, have been well documented (reviewed in Wright and Jones 2006).

Mosses can have key effects on biogeochemical processes (Turetsky 2003, Cornelissen et al. 2007, Gornall et al. 2007) and increasingly have been demonstrated to influence ecosystems at a large scale (Rocheffort 2000, Sedia and Ehrenfeld 2003, Nilsson and Wardle 2005). In acidic peatland habitats, *Sphagnum* L. moss species have been considered ecosystem engineers because of their effects on pH, organic matter accumulation, soil moisture, nutrient availability, and therefore the growth and composition of vascular plant species (van Breemen 1995, Malmer et al. 2003). Interest in the biogeochemical effects of non-*Sphagnum* moss species is of relatively recent origin, however, and surprisingly little is known about the biogeochemical role of mosses even in systems where they form a dominant part of the vegetation. For

example, rich fens (peatlands fed primarily by mineral-rich groundwater) frequently have a layer of "brown" mosses (typically in the family *Amblystegiaceae*) covering the soil surface, but little is known about moss effects on soil processes in these wetlands. Because many small-statured fen forb species have shallow root systems that penetrate only the upper centimeters of the soil, the influence of mosses on shallow soils may be particularly relevant to plant growth and therefore species composition in these unusually diverse systems (Bedford and Godwin 2003).

The effects of mosses on biogeochemical processes in fen soils are also of particular interest given the low nutrient status of rich fens, which may influence plant species composition and diversity significantly (Bedford et al. 1999, Bedford and Godwin 2003). Rich fens tend to be low in nitrogen and phosphorus, and a low concentration of phosphorus in plant-available forms often limits plant growth (Bedford et al. 1999). In rich fens, inorganic phosphate concentrations in particular may be low because phosphorus tends to occur in forms that plants cannot access easily. For example, phosphorus in rich fens may be sequestered by calcite (Boyer and Wheeler 1989, Almendinger and Leete 1998a, b), particularly at pHs greater than 7(-7.5) (Brady and Weil 2002), which are typical of fens. Phosphorus in rich fens also may be bound to iron in its oxidized form (Fe^{3+}) and released only under reducing conditions where iron instead is sequestered in iron sulfide compounds (Smolders et al. 2006). Analysis of groundwater flow patterns in relation to groundwater and soil chemistry pointed to iron oxidation-reduction processes as dominant controls on phosphorus availability for three rich fens in New York (Bailey 2006, Boomer and Bedford 2008a, b). Finally, phosphorus in rich fens may be stored in the large soil organic matter pool, from which it may be accessed through phosphatase enzyme activity by plant roots, free soil microbes, or arbuscular mycorrhizal fungi (AMF), which may provide phosphorus to plants from mineral-bound as well as organic forms

(Aerts and Chapin 2000, Vance et al. 2003, Weishampel 2005, Weishampel and Bedford 2006).

Mosses affect biogeochemical conditions in several ways relevant to phosphorus availability (Cornelissen et al. 2007). Like the *Sphagnum* species that dominate acidic peatlands, brown mosses in rich fens may decrease the pH of their immediate environment through a high cation exchange capacity (Glime et al. 1982, Bates 2000), which has implications for the pH-dependent process of calcite sequestration. Mosses insulate the soil surface (Sveinbjornsson and Oechel 1992, Gornall et al. 2007), increase or decrease soil moisture levels (Longton 1992, Beringer et al. 2001, Gornall et al. 2007), and increase organic matter content in the soil (Vitt 2000, Sedia and Ehrenfeld 2005), which in turn may influence redox potential, microbial activity, and nutrient availability (Sedia and Ehrenfeld 2005, 2006, Gornall et al. 2007, Loeffler et al. 2008). Mosses also sequester available nutrients (Li and Vitt 1997, Bates 2000), and they use phosphatase enzymes to access phosphorus from organic matter directly (Turner et al. 2001). Overall, mosses clearly affect physical and chemical conditions in ways that might be expected to influence phosphorus availability. Little is known, however, about the ways in which rich fen mosses influence phosphorus cycling, which may have significant implications for vascular plant species composition and distribution in these diverse wetlands.

To investigate the effects of rich fen mosses on phosphorus cycling, I established a moss removal experiment in three New York fens. I asked the following questions:

1. How do mosses influence physical and chemical conditions (*e.g.*, pH, soil moisture, redox potential) that might affect phosphorus availability in shallow fen soils?

2. How do mosses influence levels of available phosphate near the soil surface and corresponding phosphorus uptake by shallowly rooted forb species, which comprise much of the high diversity of these systems?
3. How do mosses influence biological activity (*e.g.*, microbial activity, root and soil phosphatase activity) that may immobilize or release phosphorus in fen soils?

Redox processes associated with groundwater flow patterns have been shown to control broad patterns of phosphorus availability across fen sites (Boomer and Bedford 2008a). Because mosses may influence underlying soil moisture, which in turn determines soil redox status, I focused on two redox-driven alternative pathways by which mosses might affect shallow soils. First, I hypothesized that mosses might create more oxidized conditions through increased evaporation relative to bare peat (Beringer et al. 2001, Heijmans et al. 2001, Heijmans et al. 2004). If this were true, phosphate availability could be correspondingly lower in soils underlying mosses (as reduced iron (Fe^{2+}) was oxidized to Fe^{3+} , which could bind phosphate, or as greater microbial activity immobilized phosphate). Vascular plant and microbial communities then might respond to lower phosphate availability with increased mycorrhizal and phosphatase enzyme activity to access phosphorus from other pools, particularly the organic pool. Because of the lower availability of readily accessible phosphate, plant tissue phosphorus concentrations likely would be lower where mosses occurred. Alternatively, I hypothesized that the high water-holding capacity of mosses (Clymo and Hayward 1982, Proctor 2000) might generate more reducing conditions. If this were true, phosphate availability could be correspondingly higher (as Fe^{3+} was reduced to Fe^{2+} , releasing phosphate, or due to lower phosphorus demand), vascular plants and microbial communities might demonstrate less use of phosphatase enzymes or other alternative acquisition pathways, and plant tissue phosphorus concentrations likely would be higher where mosses occurred. Under either pathway, such a

sequence of moss effects would demonstrate an important influence by rich fen mosses on rooting conditions for a diverse fen flora.

METHODS

Study sites

I established a moss removal experiment at three rich fens near Cortland, New York (42.6°N, 76.2°W): Larry's Fen (LF), Fish Fen (FF), and Clark Street Extension Fen (CSE). Larry's Fen is sloping and fed by hillside seepage, with vegetation dominated by a matrix of *Carex* L. (sedge) species, *Dasiphora floribunda* (Pursh) Raf. (shrubby cinquefoil), mixed forbs, and the moss species *Campylium stellatum* (Hedw.) C. Jens. Clark Street Extension Fen has a similar plant species composition to Larry's Fen but slopes more gently and is dominated by *Campylium stellatum* and *Sphagnum warnstorffii* Russ. in the moss layer. Fish Fen is a sloping depressional fen dominated by *Typha angustifolia* L. (narrowleaf cattail), diverse forbs, and the moss species *Calliergonella cuspidata* (Hedw.) Loeske and *Campylium stellatum* plus a number of less frequent mosses. (Nomenclature follows USDA and NRCS (2009).) The moss layers (which include a minimal abundance of liverwort species) ranged in depth from approximately 3-5 cm and ranged in coverage from nearly continuous (85-95% cover) to highly patchy (20-90% cover) within and among the three sites.

LF and CSE occur within predominantly agricultural watersheds, and CSE has a known history of past cattle grazing. While FF is surrounded mainly by steeply sloping forest and is located within a natural area, it served as a millpond during the late 1800s and has accumulated a minimum of 25-35 cm of new peat since abandonment. At all three sites, water levels remained within 5-10 cm of the peat surface throughout the growing season. Table 1-1 summarizes physical and chemical characteristics of the three sites.

Table 1-1. Selected groundwater and soil chemistry characteristics¹ for Clark Street Extension Fen (CSE), Fish Fen (FF) and Larry's Fen (LF) (mean (SD), n=5 except where noted).

Site	pH	Conductivity (uS cm ⁻¹)	Bulk density (g cm ⁻³)	N (%)	C (%)	P (ug g ⁻¹)	Ca (ug g ⁻¹)	Fe (ug g ⁻¹)
CSE	6.9 (0.16)	556 (51)	0.10 (0.02)	2.5 (0.2)	41 (3)	977 (47)	28295 (3481)	17159 (26260)
FF	7.4 (0.18)	428 (18)	0.06 (0.01)	2.2 (0.1)	39 (3)	1103 (200)	25184 (1503)	4212 (2402)
LF	7.0 (0.24)	551 (54)	0.12 (0.01)	2.0 (0.2)	26 (1)	1067 (372)	21854 (6149)	14881 (5619)

¹Conductivity and pH measurements were taken at a depth of 1 cm below the peat surface. Bulk density estimates were based on 10-cm core sections collected in 2006-2007 (n=4 for CSE and n=3 for FF and LF). Soil N, C, P, Ca, and Fe were based on data from 1998-99 (Bailey 2005) for FF and LF and from 2008 for CSE, analyzed via C:N analyzer and ICP at Cornell Nutrient Analysis Laboratories, Ithaca, NY.

Experimental design

Beginning in June 2005 at LF and FF and in June 2006 at CSE, I established five sets of paired 0.5x0.5-m randomly assigned moss removal and moss control plots at each fen. Treatments within each pair included a moss control plot with no manipulation and a moss removal plot in which mosses were removed while all other plants (predominantly forbs and graminoids) were left in place. In removal plots, all living mosses were removed by hand, using a definition of living moss as green tissue (ranging from 2-3 cm depth) plus firmly attached brown tissue (1-2 cm). Because living mosses separated cleanly from the peat at this boundary, the peat surface could be identified consistently in moss control and moss removal plots, ensuring that subsequent water and soil chemistry measurements would be comparable between treatments. Treatment blocks were established in regions of each fen where the dominant moss species were brown mosses, predominantly *Campylium stellatum* or *Calliergonella cuspidata*, with a minimum total moss cover of 80%. The moss removal treatment was maintained twice per growing season to counteract moss recolonization.

In each treatment block at LF and FF, I also established a disturbance control plot in which mosses were removed and replaced (in place) to ensure that the effects of moss removal were not caused by soil disturbance. Analyzed across the 2006 growing season, measures of available phosphate did not differ significantly between control and disturbance control plots at LF but had not yet equilibrated at FF (Appendix), suggesting that FF treatment blocks required additional equilibration time after treatment application. I therefore am presenting results for all three sites from summer 2007, supplemented by data collected in fall 2006 at LF.

Soil chemistry

In June and August 2007, I measured available inorganic phosphate (PO_4^{3-}) in shallow soils using anion exchange membrane (AEM) resin strips to measure as closely as possible the PO_4^{3-} to which plant roots would be exposed in the soil zone most affected by mosses (Qian and Schoenau 2002). I installed three 2.5x5-cm AEM strips per plot (GE Water & Process Technologies AR204SZRA, Watertown, MA) for four-week periods parallel to the peat surface at a depth of 1 cm. Prior to installation, resin strips were eluted with 0.5 M hydrochloric acid (HCl) and charged with 0.5 M sodium bicarbonate (NaHCO_3) solution changed hourly for 5 hours. Upon collection from the field, resin strips were scrubbed gently under deionized water, bulked by plot, and extracted with 17.5 mL 0.5 M HCl per resin strip (Western Ag Innovations Inc. 2004). Resin extracts were stored at 35°C until analysis, and analyzed for PO_4^{3-} with a modified Murphy-Riley colorimetric method (Lajtha et al. 1999) using a LaMotte SMART Spectrophotometer.

Also in June and August 2007, I measured water depth below the peat surface in shallow pits and *in situ* oxidation-reduction potential (ORP) at a depth of 1 cm using an Orion 250A meter. As a second indicator of redox potential, I measured reduced iron (Fe^{2+}) concentrations in water samples extracted from a soil depth of 1 cm using a 60-mL syringe with attached Teflon tubing. I analyzed Fe^{2+} concentrations colorimetrically (absorbance = 562 nm) for 5 mL subsamples filtered (Whatman GF/B) immediately into 5 mL of 50 mM HEPES buffer solution with 2 mM ferrozine (adapted from Stookey 1970, Lovley and Phillips 1987). I also used a YSI 63 meter to measure pH and conductivity for 30 mL subsamples.

In May 2007 at FF and LF, I collected soil cores 5 cm in diameter and 10 cm in depth from four moss control and four moss removal plots at each site for analysis of organic phosphorus pools. Cores were shipped on ice the day of collection to

Alexander Cheesman, University of Florida Soil & Water Science Department.

Mosses then were removed from control cores, cores were weighed for bulk density estimates, samples were alkaline-extracted with sodium hydroxide (NaOH), and homogenized samples (by treatment and site) were analyzed for organic phosphorus (including monoesters and diesters) through solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Microbial phosphorus also was determined for each core individually using an AEM-hexanol fumigation-extraction procedure (Cheesman personal communication).

Phosphatase enzyme activity

In June 2007 at FF and LF, I measured soil phosphatase activity on three homogenized soil subsamples per plot from a depth of 0-2 cm, from which I removed roots >1 mm in diameter and rhizomes with attached roots. Measurements of soil phosphatase activity therefore included soil microbial phosphatase activity plus activity by remaining small root segments. I measured monoesterase and diesterase activity colorimetrically using para-nitrophenyl phosphate (*p*NPP; Sigma P4744) and bis-para-nitrophenyl phosphate (bis-*p*NPP; Sigma 123943) as substrates (modified from Tabatabai 1982). Each monoesterase or diesterase analysis for a site included two assay replicates per soil sample (1 g fresh soil + 4 mL 50 mM PIPES buffer + 2 mL 7.5 mM *p*NPP or bis-*p*NPP in 50mM PIPES buffer), one corresponding sample control per soil sample (1 g soil + 6 mL 50mM PIPES buffer), and three substrate controls (4 mL 50 mM PIPES buffer + 2 mL 7.5 mM *p*NPP or bis-*p*NPP in 50mM PIPES buffer). PIPES buffer was adjusted to pH 7.2 before use to match a typical fen pH. All tubes were incubated on a shaker table at 100 rpm in a 20°C dark water bath for 45 minutes. I then centrifuged the tubes for five minutes at 2300 rpm and pipetted 5 mL of the supernatant through Whatman 2V filter paper into 5 mL of terminator

solution (200 mM NaOH, 5 mM EDTA, 100 mM K₂HPO₄). The terminator solution stopped the reaction and caused the yellow *p*NP color to develop without causing bis-*p*NPP to dissociate (Turner et al. 2001). Absorbance was measured at 410 nm on a LaMotte SMART spectrophotometer, and absorbance for the sample and substrate controls was subtracted from assay absorbance to correct for interference.

At LF in May 2006, I planted four-week-old seedlings of *Carex hystericina* Muhl. ex Willd. (bottlebrush sedge), *Solidago patula* Muhl. ex Willd. (roundleaf goldenrod), and *Lycopus uniflorus* Michx. (northern bugleweed) in moss control and moss removal plots, placing the base of the plant at the peat surface in both plot types, to provide an index of root phosphatase activity. In September 2006, I harvested surviving seedlings, cleaned the roots with deionized water, and soaked them for 30 seconds in 1 mM Fe-EDTA (sodium salt)-0.01% sodium azide solution to kill surface microorganisms (Kuhn et al. 2002). I then measured root monoesterase activity on 0.05-0.1-g root samples (fresh weight). Each run of 10 root samples included one assay (root sample + 4 mL 50 mM PIPES buffer + 2 mL 7.5 mM *p*NPP in 50 mM PIPES) and one corresponding sample control (root sample + 6 mL 50 mM PIPES buffer) per root sample. Each run also included three substrate controls (4 mL 50 mM PIPES buffer + 2 mL 7.5 mM *p*NPP in 50 mM PIPES). Tubes were incubated in a 20°C dark water bath for one hour, and the supernatant was filtered through Whatman 2V filter paper into 2 mL of 0.5 M NaOH to stop the reaction prior to measurement of absorbance at 410 nm.

Microbial biomass and respiration rates

For FF and LF in June 2007, I measured soil microbial respiration rates for moss control and moss removal plots and subsequently measured microbial biomass using a substrate-induced respiration (SIR) method (Anderson and Domsch 1978,

West and Sparling 1986, Fisk et al. 2003). The SIR method determines maximum soil microbial respiration rates following addition of labile carbon to soil samples; these maximum rates are considered proportional to the original microbial biomass of the soil (Anderson and Domsch 1978, West and Sparling 1986). To measure initial microbial respiration rates and apply the SIR method, I first homogenized three soil subsamples per plot from a depth of 0-2 cm and removed large rhizomes and roots >1 mm in diameter. Within 24 hours of collection, I sealed two soil samples per plot (2 g dry weight/sample) in paired 228-mL glass mason jars under aerobic conditions. I used infrared gas chromatography to measure carbon dioxide (CO₂) production in the jar headspace after 2, 6, 12, and 24 hours to assess initial microbial respiration rates. After seven days, I added 20 mL of deionized water to one jar in each pair and an equivalent volume of glucose solution to the second jar to achieve 4.0 mg carbon per gram dry soil. Headspace CO₂ concentrations in the water- and glucose-amended jars were measured 2 and 6 hours after jars were closed to provide estimates of basal respiration and SIR, respectively. Incubations were continued after 12 hours and at further 12-hour intervals until respiration rates equilibrated between jars in each pair. Microbial biomass carbon was calculated from measures of SIR following West and Sparling (1986).

Mycorrhizal colonization

For LF in September 2006, I assessed colonization by arbuscular mycorrhizal fungi (AMF) of *Solidago patula* seedlings in moss control and moss removal plots, planted and harvested as described above, with roots preserved until analysis in 50% ethanol. Roots were subsampled and cut into 1-2 cm lengths, cleared with 10% KOH (Vierheilig et al. 1998), and stained using Lacto-Cotton Blue (Sime et al. 2002). I

quantified colonization by AMF using a line-intersection method (McGonigle et al. 1990) and calculated percent colonization by fungal hyphae, vesicles, and arbuscules.

Plant tissue chemistry

For seedlings of *Solidago patula*, *Lycopus uniflorus*, and *Carex hystericina* harvested in September 2006, I dried shoot tissues at 60°C to constant mass. To analyze tissue phosphorus concentrations, I digested dried shoots, apple-leaf Standard Reference Material (NIST SRM 1515), and blanks (to check for phosphorus contamination during digestion) in a heated aluminum block in 1.5 mL concentrated sulfuric acid (H₂SO₄) with hydrogen peroxide (H₂O₂) (Allen 1989, Weishampel 2005). I added 2 mL of H₂O₂ in 0.5 mL aliquots, raised the temperature to 320 degrees C over a 90-minute period, and held this temperature for 20 minutes to boil away un-reacted H₂O₂. I then allowed the samples to cool and repeated the H₂O₂ additions if needed to achieve a colorless digest. Cooled digests were diluted to 25 mL, and samples, apple-leaf standards, and blanks were analyzed for total phosphorus through inductively coupled plasma (ICP) spectroscopy at the Cornell Nutrient Analysis Laboratory, Ithaca, NY.

Data analysis

I analyzed data from the moss removal experiment using generalized linear mixed models (proc MIXED, SAS version 9.1). For measurements taken in June and August 2007, I analyzed treatment effects on each response variable (PO₄³⁻-P, Fe²⁺, etc.) using a repeated measures analysis in which block was a random factor, site and treatment were fixed factors, and month was the repeated measure for each site-block-plot combination. Where treatment interacted with site or sampling month, I used contrasts to examine the effect of treatment given each site or time step. For data

collected at a single time step, I analyzed treatment effects on each response variable using a 3-way model in which block was a random factor and site and treatment were fixed factors (without the repeated measure). Where needed, I log-transformed variables to satisfy model assumptions of normality and homogeneity of variance, and I used Satterthwaite's approximation to estimate denominator degrees of freedom (Littell et al. 2006). Finally, I used a Spearman rank order correlation analysis to assess the strength of the non-linear relationships between phosphorus availability and other variables.

RESULTS

Physicochemical conditions

Mosses clearly influenced physical and chemical conditions in shallow fen soils. In June and August 2007, oxidation-reduction potential (ORP) was greater and the concentration of reduced iron (Fe^{2+}) correspondingly lower in moss control plots than where mosses had been removed, at a depth of 1 cm below the peat surface (Figure 1-1). This difference was consistent across sites and significant at Larry's Fen (LF) and Clark Street Extension Fen (CSE). Although moss removal significantly affected redox conditions in shallow soils, it did not affect water depth below the peat surface (Figure 1-2), which might be expected to determine soil redox status; however, a trend toward greater mean water depth in moss control than in moss removal plots (Figure 1-2) suggested that drier surface soils underlying mosses could be driving observed patterns in redox potential. Volumetric soil moisture content at Fish Fen (FF) was significantly lower underlying mosses than where they had been removed, supporting this possibility, but volumetric soil moisture did not differ between treatments at LF (Figure 1-3). Conductivity and pH at a depth of 1 cm below the peat surface showed no effect of moss removal (data not shown).

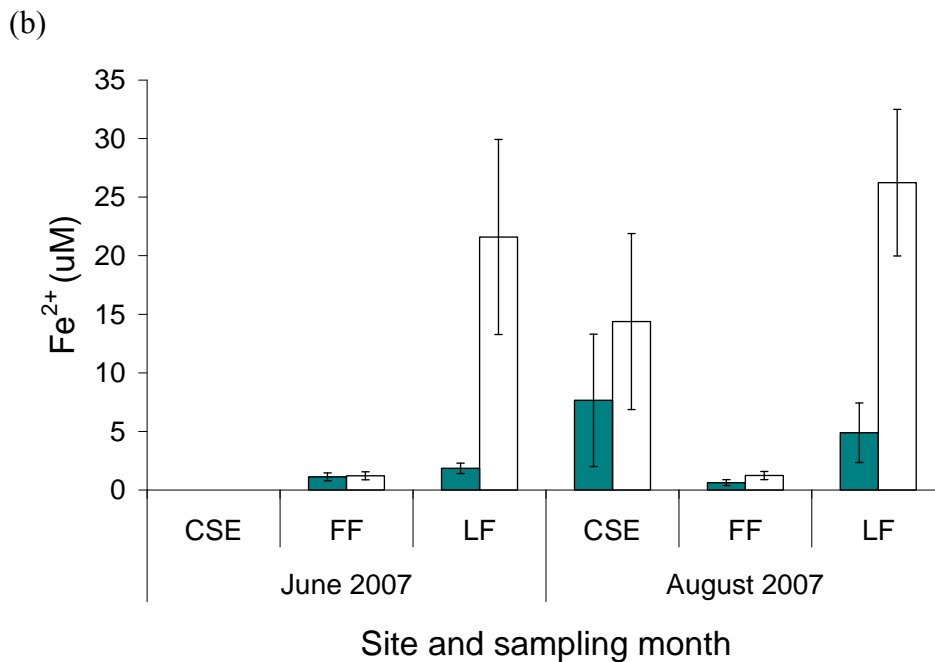
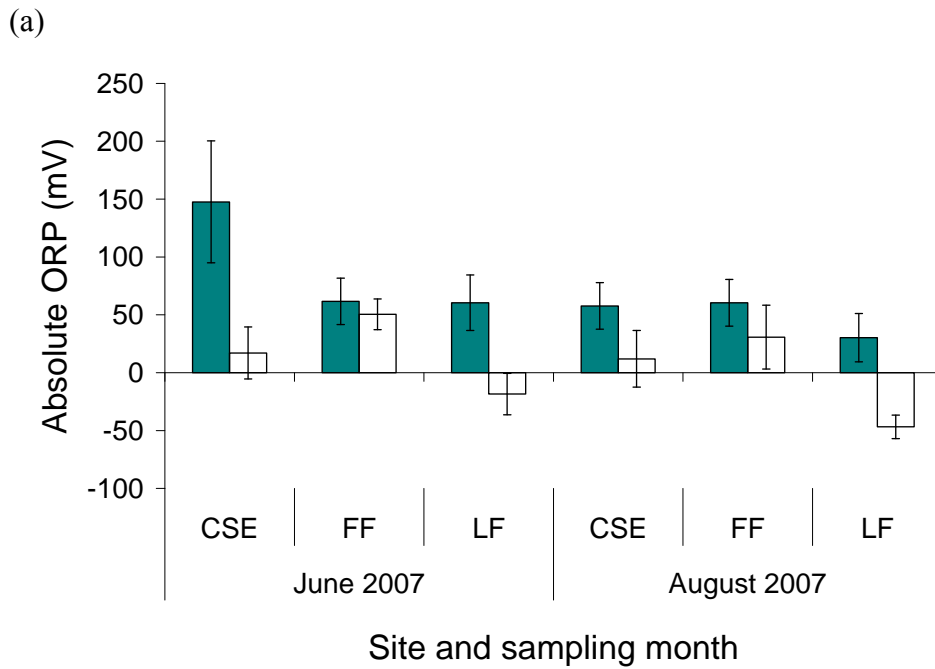


Figure 1-1. Indicators of redox potential for moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF), Larry's Fen (LF), and Clark Street Extension Fen (CSE). Greater redox potential (more oxidized conditions) in moss control plots was indicated by (a) greater oxidation-reduction potential (ORP) (CSE, $p=0.0003$; FF, n.s.; LF, $p<0.0001$), and (b) a correspondingly lower concentration of reduced iron (Fe^{2+}) (CSE, $p=0.01$; FF, n.s.; LF, $p<0.0001$). Error bars \pm SE, $n=5$.

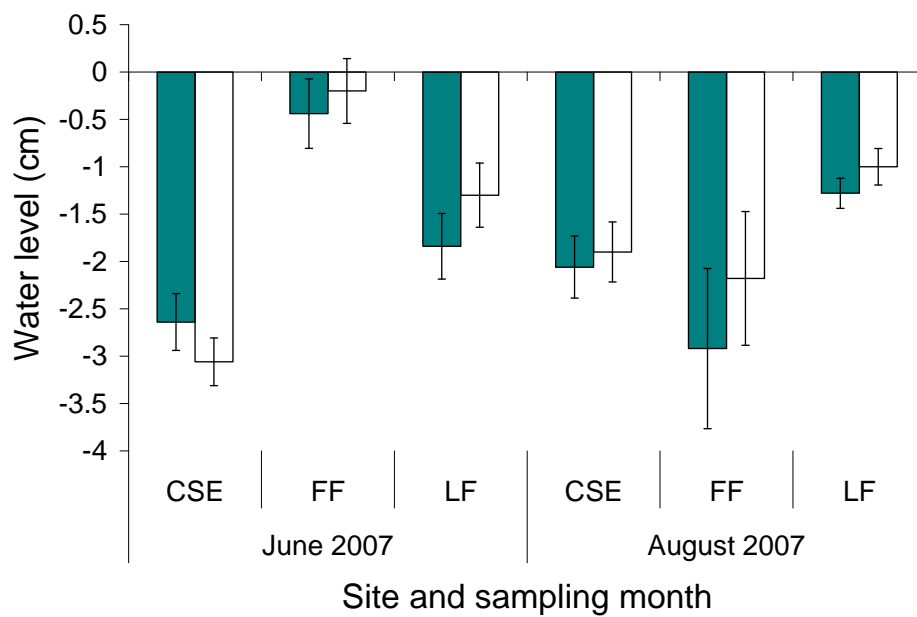


Figure 1-2. Differences in depth of water below the peat surface between moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF), Larry's Fen (LF), and Clark Street Extension Fen (CSE) in June and August 2007 (n.s.). Error bars +/- SE, n=5.

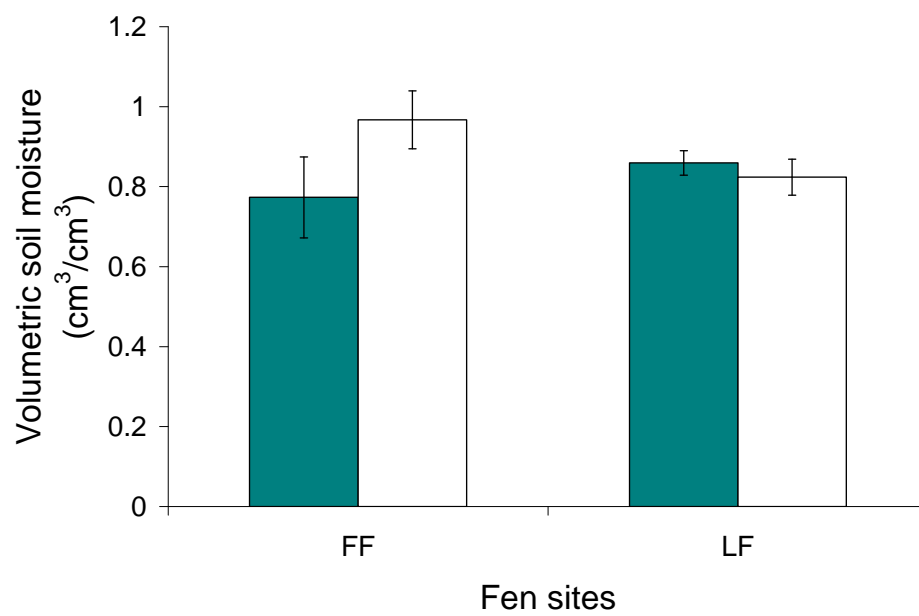


Figure 1-3. Differences in volumetric soil moisture (FF, $p < 0.001$; LF, n.s.) between moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF) and Larry's Fen (LF) in June 2007. Error bars \pm SE, $n=5$.

Soil phosphorus availability

Across sites, phosphorus supplied as available phosphate ($\text{PO}_4^{3-}\text{-P}$) to anion exchange membrane (AEM) resin strips was lower in shallow soils underlying mosses than where mosses had been removed (Figure 1-4). Total $\text{PO}_4^{3-}\text{-P}$ and the magnitude of the difference in availability between treatments were greatest in August 2007, when $\text{PO}_4^{3-}\text{-P}$ in moss control plots was 20-60% of levels in moss removal plots. Lower $\text{PO}_4^{3-}\text{-P}$ underlying mosses corresponded to the greater redox potential measured at an identical depth below the peat surface in the same sampling periods. Across sites and sampling times, $\text{PO}_4^{3-}\text{-P}$ was negatively correlated with ORP and positively correlated with Fe^{2+} (Table 1-2). Analyzed by site and month, similar correlations between phosphorus availability and indicators of redox potential were significant for LF in June and August and for FF in June (Table 1-2).

While levels of phosphate in shallow soils responded to the moss removal treatment, after two years the amount of organic phosphorus (specifically monoesters and diesters) at LF and FF did not differ in the upper 10 cm of soil between moss control plots and areas where mosses had been removed (Figure 1-5). The abundance of monoesters was 2-3 times the level of diesters, however (Figure 1-5). Additionally, while the abundance of diesters was very similar between sites, the abundance of monoesters was approximately 40% higher at LF than at FF (Figure 1-5).

Microbial and vascular plant responses

In addition to effects on soil chemistry, the presence of mosses significantly influenced biological activity belowground. Both initial microbial respiration rates and microbial biomass carbon at LF and FF were greater underlying mosses than where mosses had been removed (Figure 1-6). Across sites and sampling times, microbial biomass was negatively correlated with $\text{PO}_4^{3-}\text{-P}$ supplied to AEM resin

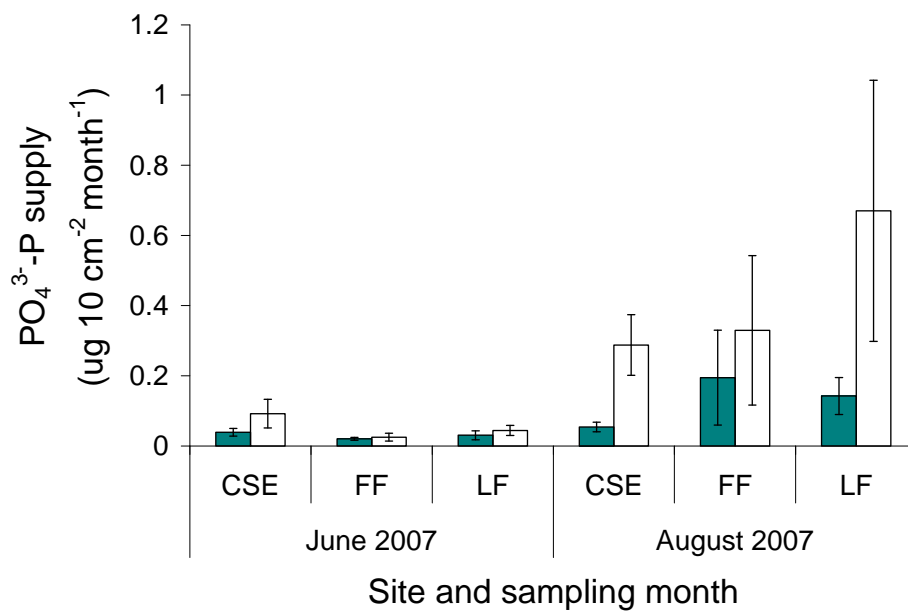


Figure 1-4. Differences in phosphorus supplied as available phosphate ($\text{PO}_4^{3-}\text{-P}$) to anion exchange membrane (AEM) resin strips at 1 cm below the peat surface between moss control (solid bars) and moss removal plots (open bars) (June, n.s.; August, $p=0.0003$) at Fish Fen (FF), Larry's Fen (LF), and Clark Street Extension Fen (CSE). Error bars +/- SE, $n=5$.

Table 1-2. Spearman rank order correlation coefficients between (a) phosphorus supplied as phosphate ($\text{PO}_4^{3-}\text{-P}$) to anion exchange membrane (AEM) resin strips and (b) microbial biomass carbon (C) or indicators of redox potential (including reduced iron (Fe^{2+}) and oxidation-reduction potential (ORP)). Analyses were performed at three levels of organization: (1) across site and time; (2) by month, across sites; and (3) by each month-site combination. Significance indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; + $p < 0.1$. FF = Fish Fen, LF = Larry's Fen.

Level of analysis	Month	Site	n	Microbial		
				biomass C	Fe^{2+}	ORP
Across site and time	June & August	FF & LF	40	-0.31*	0.32*	-0.42**
	June	FF & LF	20	-0.32	0.66**	-0.51*
By month, across sites	August	FF & LF	20	-0.59**	0.29	-0.35
	June	FF	10	-0.01	0.71**	0.07
By site and month	June	LF	10	-0.16	0.54 ⁺	-0.84**
	August	FF	10	-0.75*	0.01	0.03
	August	LF	10	-0.45	0.36	-0.56 ⁺

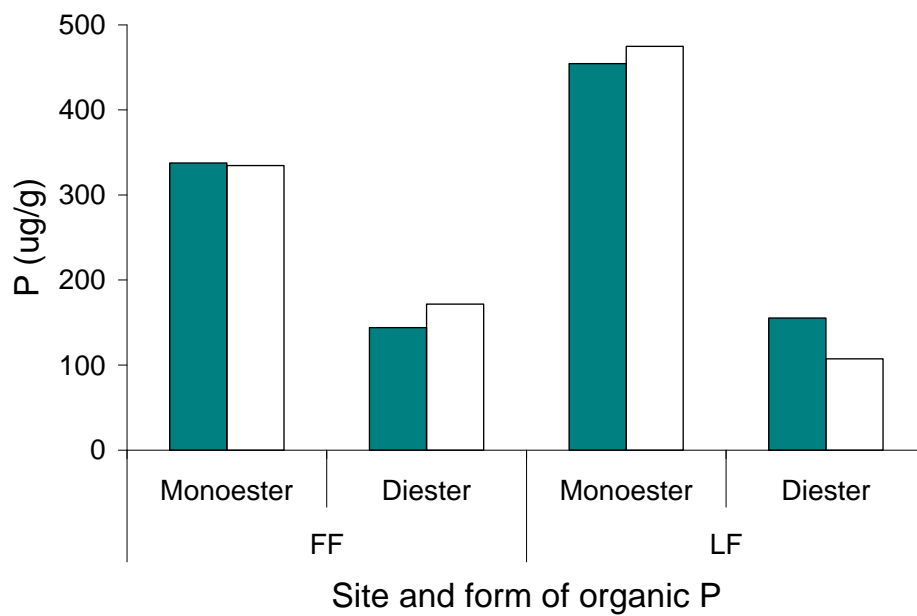


Figure 1-5. Differences in organic phosphorus concentrations (monoesters or diesters) between moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF) and Larry's Fen (LF) in May 2007, after two years of moss removal. Measured on composites of four samples using solution ^{31}P NMR spectroscopy; data are from Alexander Cheesman, University of Florida.

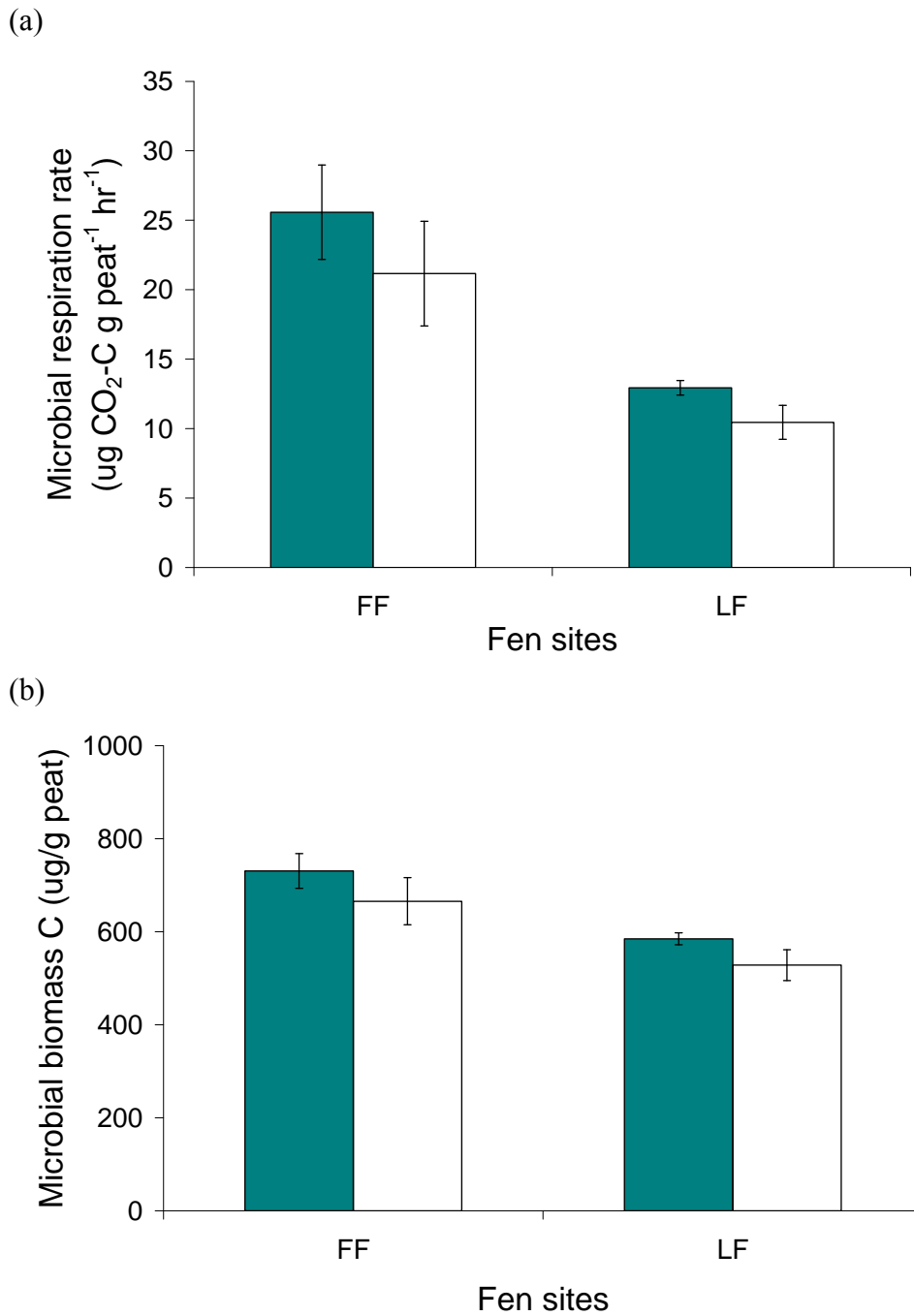


Figure 1-6. Differences in (a) initial microbial respiration rates ($p < 0.0001$) and (b) microbial biomass carbon derived from SIR ($p = 0.02$) between moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF) and Larry's Fen (LF) in June 2007. Error bars \pm SE, $n = 5$.

strips. Analyzed by site and month, the negative correlation between $\text{PO}_4^{3-}\text{-P}$ and microbial biomass was significant for FF in August (Table 1-2).

At LF and FF in June 2007, soil monoesterase activity was 25-50% greater immediately underlying mosses than where mosses had been removed (Figure 1-7), as expected given the lower $\text{PO}_4^{3-}\text{-P}$ supplied to AEM resins underlying mosses. While soil diesterase activity showed a similar trend, the difference in diesterase activity between treatments was not significant (Figure 1-7). At both sites, the level of soil monoesterase activity was approximately twice that of soil diesterase activity, matching the differences in abundance between monoesters and diesters at the sites (Figure 1-5, 1-7).

Like soil monoesterase activity, root monoesterase activity for seedlings of the forb species *Solidago patula* and *Lycopus uniflorus* harvested at LF in September 2006 was 30-50% greater in moss control than in moss removal plots (Figure 1-8). In contrast, for the sedge species *Carex hystericina*, root phosphatase activity showed no response to moss removal (Figure 1-8). Colonization by arbuscular mycorrhizal fungi (AMF) of *Solidago patula* seedlings at LF responded to the moss removal treatment in the same direction as did phosphatase enzyme activity: AMF root colonization was twice as high for seedlings planted in moss control plots (Figure 1-9). Greater root monoesterase activity and AMF colonization for moss control plots also corresponded to lower levels of $\text{PO}_4^{3-}\text{-P}$ supplied to AEM resin strips in 2006 (Crowley and Bedford, unpublished data).

Unexpectedly given the lower levels of $\text{PO}_4^{3-}\text{-P}$ measured where mosses occurred, microbial phosphorus was greater for soils underlying mosses than where mosses had been removed (Figure 1-10). Additionally, plant tissue phosphorus concentrations were greater for seedlings of the two forb species planted in moss control plots (Figure 1-11); again, the sedge species *Carex hystericina* showed no

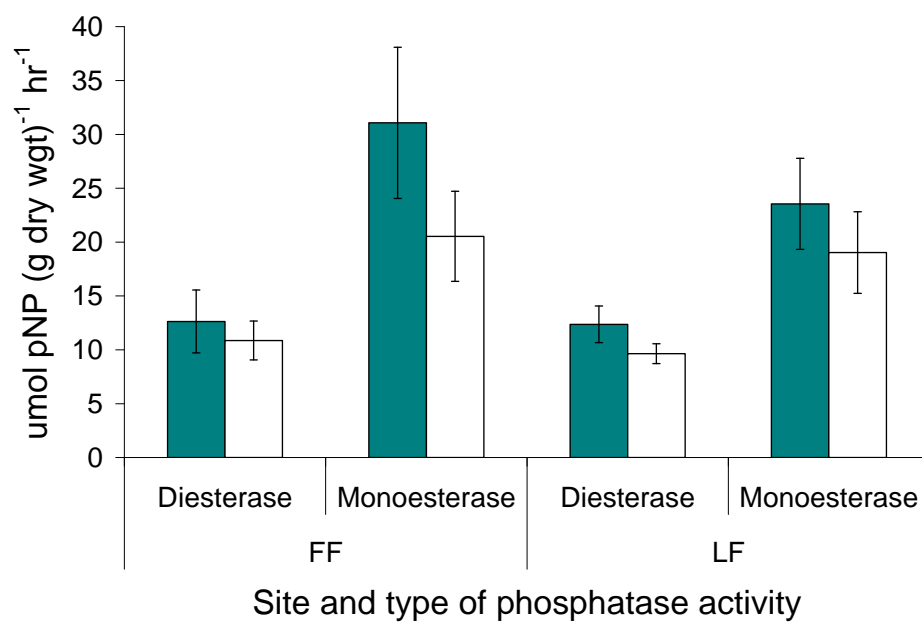


Figure 1-7. Differences in soil monoesterase ($p=0.06$) and diesterase (n.s.) activity between moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF) and Larry's Fen (LF) in June 2007. Error bars \pm SE, $n=5$.

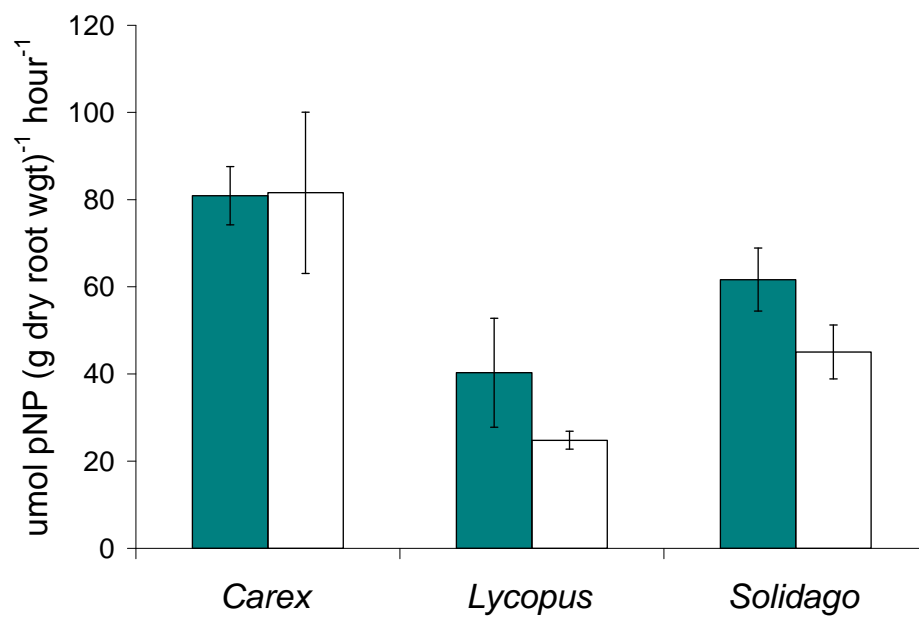


Figure 1-8. Differences in root monoesterase activity ($p=0.05$) between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina*, *Lycopus uniflorus*, and *Solidago patula* at Larry's Fen (LF) in September 2006. Error bars \pm SE, $n=5$.

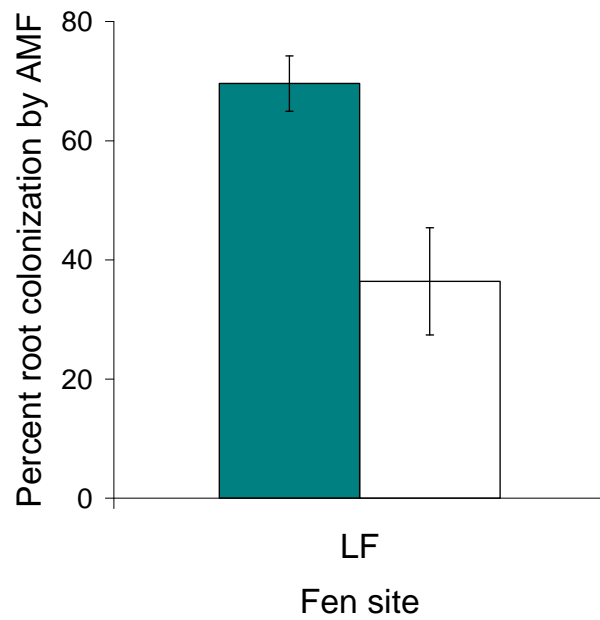


Figure 1-9. Difference in percent root colonization by arbuscular mycorrhizal fungi (AMF, hyphae, vesicles, and arbuscules; $p=0.02$) between moss control (solid bar) and moss removal plots (open bar) for *Solidago patula* test seedlings at Larry's Fen (LF) in September 2006. Error bars \pm SE, $n=5$.

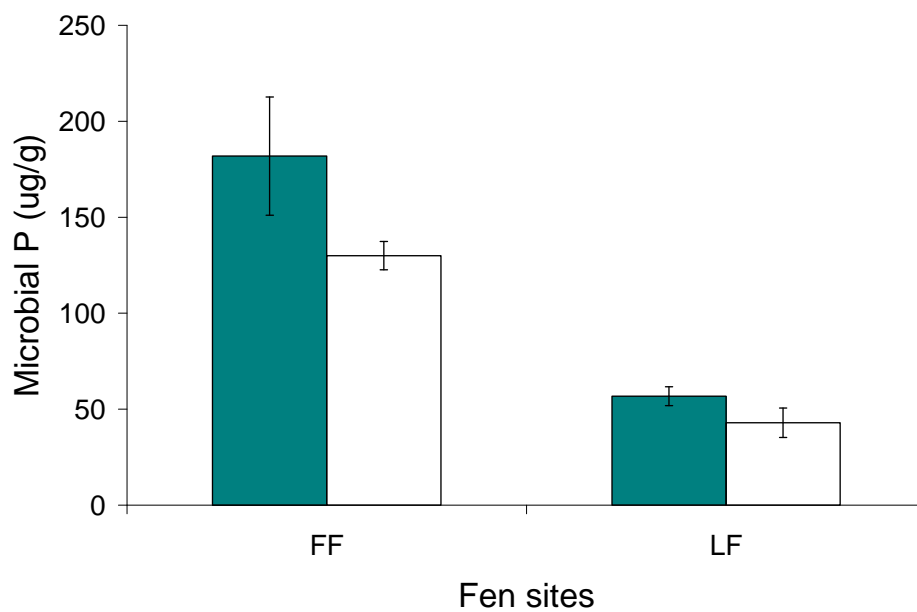


Figure 1-10. Differences in microbial phosphorus ($p=0.05$) between moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF) and Larry's Fen (LF) in May 2007. Error bars \pm SE, $n=5$. Data are from Alexander Cheesman, University of Florida.

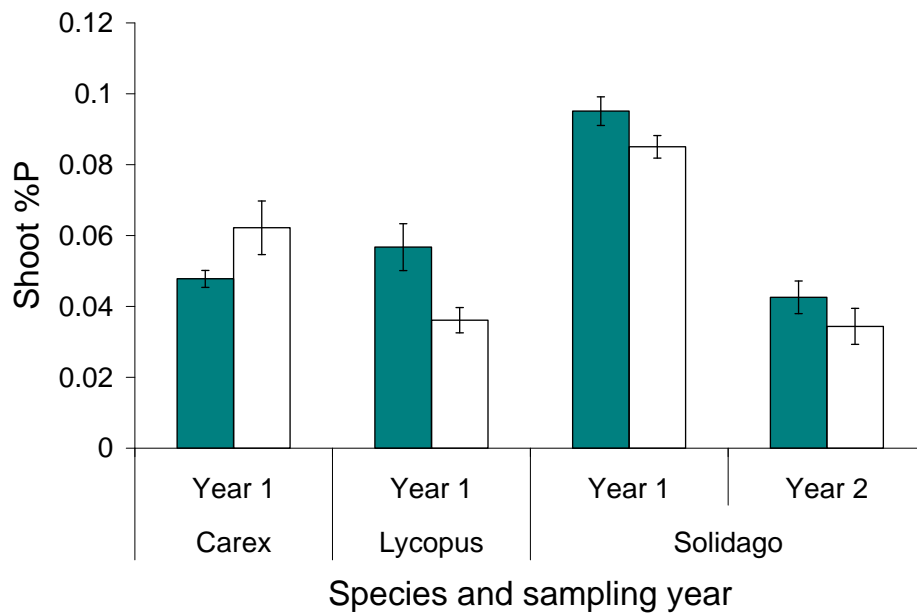


Figure 1-11. Differences in shoot tissue phosphorus concentrations between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina* (n.s.), *Lycopus uniflorus* ($p < 0.01$), and *Solidago patula* ($p < 0.05$ across years) at Larry's Fen (LF), harvested after one or two growing seasons (indicated as year 1 or year 2). Error bars \pm SE, $n=5$.

response to moss removal. Greater microbial phosphorus and forb tissue phosphorus concentrations where mosses occurred suggested that phosphorus could be more available for uptake from moss-covered than from moss-free soils, counter to expectations based on lower $\text{PO}_4^{3-}\text{-P}$ supply to AEM resins.

DISCUSSION

Field manipulation of mosses significantly affected redox conditions and phosphorus availability in shallow fen soils. Mosses also influenced the soil biological community, stimulating microbial activity, soil and forb root phosphatase enzyme activity, and colonization of forb roots by arbuscular mycorrhizal fungi (AMF). Greater redox potential underlying mosses, with associated chemical and biological responses to more oxidized conditions, cumulatively may have resulted in greater total phosphorus uptake by forb species where mosses occurred. This suggests the importance of rich fen mosses both in alleviating stress from saturated soils and in cycling phosphorus, which frequently limits growth of shallowly rooted forbs in these systems.

Effects of mosses on physicochemical conditions

Experimental moss removal demonstrated that where mosses occurred, redox potential was significantly greater in shallow soils (Figure 1-1), indicating more oxidized and potentially drier conditions underlying fen mosses. This result initially may appear counter-intuitive, as mosses often are considered to increase soil moisture (Longton 1992), possibly creating a waterlogged condition that instead might be expected to reduce redox potential. *Sphagnum* species with their high water-holding capacity, for example, may cause waterlogging even leading to wetland formation (Longton 1992, Vitt and Kuhry 1992). Thick moss cover may reduce evaporation

because of the lack of vascular tissue and the consequent limitation of water movement to short distances above the soil surface; in a study in Barrow, Alaska, only the shallowest moss layers (≤ 2 cm) showed water movement from the soil to growing tissue (Miller et al. 1980). At a high arctic site in Svalbard, Norway where soil moisture instead was lower under deep relative to shallow mosses after two growing seasons, this was considered an artifact of delayed thaw under deep mosses and an associated reduction in moisture from snowmelt (Gornall et al. 2007).

In spite of the high water-holding capacity of mosses, however, moisture loss at times can be greater from a moss-covered surface than from bare peat due to the larger evaporative surface area of mosses. In saturated soils, capillary rise may be sufficient to enable upward water movement to elevations where evaporation can occur. In a wind tunnel experiment, mosses demonstrated greater evaporation with greater stem height and interruptions in the moss canopy, which allowed wind to penetrate the boundary layer of still air along the moss surface (Rice et al. 2001). Such an open growth form is typical of several rich fen moss species. In field and greenhouse settings, Heijmans et al. (2001) determined that for bog vegetation in the Netherlands, evapotranspiration was determined to a large extent by *Sphagnum* evaporation and to a lesser extent by vascular plant transpiration; the authors attributed these results most likely to a wind effect. In boreal black spruce vegetation ranging from closed forest to open bog, mosses (*Sphagnum capillifolium* or *Hylocomium splendens*) contributed 15-45% to total evapotranspiration, with the highest contribution in open wetland habitats (Heijmans et al. 2004). Additionally, when the NCAR Land Surface Model was modified to include moss effects, incorporation of mosses resulted in greater ground evaporation and reduced surface soil moisture relative to bare loam soils (Beringer et al. 2001). If evaporation from a moss-covered

surface reduced moisture in shallow soils, redox potential also would be expected to increase due to more rapid diffusion of oxygen under drier conditions.

If significant in rich fens, greater evaporation from mosses relative to bare peat could be reflected in lower soil moisture or a greater depth to water below the peat surface where mosses occurred. While differences were not statistically significant, water depth below the peat surface was consistently greater underlying mosses across all three sites in most sampling periods in 2006 and 2007 (Figure 1-2; 2006 data not shown). Additionally, volumetric soil moisture was lower in moss control than in moss removal plots at Fish Fen (FF), although no corresponding treatment difference was evident at Larry's Fen (LF) (Figure 1-3). Given the sensitivity of redox conditions to slight differences in moisture availability, even a small decrease in soil moisture underlying mosses relative to bare peat could be sufficient to generate a significant difference in redox potential.

Continuous water table elevation data collected for three rich fens in upstate New York support the possibility that more oxidized conditions underlying mosses could be generated by moss effects on evaporation and thus soil moisture (Bailey 2006). Bailey (2006) observed that while groundwater inputs maintained water table elevations consistently within 5-10 cm of the peat surface throughout the growing season, water levels fluctuated diurnally from a minimum of 2 cm to as much as 10 cm. Bailey (2006) attributed these daily water table fluctuations to greater evapotranspiration by fen vegetation during daytime hours, which suggests that the presence of vegetation can be sufficient to influence water levels even in these groundwater-fed systems. Observed moss contributions to evaporation (e.g., Heijmans et al. 2001, Rice et al. 2001); greater root biomass underlying mosses (also implying greater evapotranspiration from moss-covered areas; Chapter 2); and diurnal fluctuations in fen water table elevations (Bailey 2006) together support the possibility

that rich fen mosses could influence redox potential through diurnal, spatially variable effects on evapotranspiration and thereby soil moisture. Further study of rich fen water levels, soil moisture content, and oxygen concentrations in the presence and absence of mosses, with particular attention to timing of measurement during daily cycles of water table fluctuation, would be needed to confirm this hypothesis.

In contrast to their effects on redox potential in shallow soils, rich fen mosses had no measurable effect on pH under field conditions. *Sphagnum* species long have been considered to acidify their microenvironment through a high cation exchange capacity strong enough to affect pH at a site scale (Clymo and Hayward 1982). The brown mosses typical of rich fens have a cation exchange capacity lower than that of *Sphagnum* but high relative to other moss groups (Bates 2000), and they have been shown either to reduce (Glime 1982) or increase (Kooijman and Bakker 1994) pH in laboratory conditions, depending on the cation composition both of source habitats and of water used for the experiment. In these New York sites, moss removal did not affect field pH in shallow soils, suggesting either that cations adhering to moss exchange sites precluded H⁺ ions from being released (Kooijman and Bakker 1994), or that acidification by brown mosses may be insufficient to effect change in a well buffered fen system.

Effects of mosses on phosphorus availability in shallow soils

Phosphorus supplied as phosphate (PO₄³⁻-P) to anion exchange membrane (AEM) resin strips was as much as 60% lower where mosses occurred than where they had been removed (Figure 1-4), as expected for more oxidized underlying soils. Bryophytes can influence nutrient availability in soils via direct absorption (reviewed in Bates 2000) as well as through indirect effects; for example, mosses in an Alaskan black spruce forest were responsible for 75% of the annual phosphorus accumulation

aboveground and may have supplied phosphorus through mycorrhizal transfer to black spruce trees (Chapin et al. 1987). In rich fens, however, such direct moss uptake likely would have little influence on underlying levels of available phosphate. Rich fen moss tissue phosphorus concentrations are low relative to those of vascular plants (Bedford and Boomer, unpublished data), and living mosses in these fens occurred in a layer only 3-5 cm thick. Additionally, mosses are considered to obtain most nutrients from above, such as through atmospheric deposition or decomposing plant litter (Bates 2000). While moss uptake could prevent downward movement of phosphorus from these sources, the high demand for and low mobility of phosphorus in fen soils likely would preclude inputs from above from reaching underlying soil pools, regardless of whether mosses were present. Finally, resin strips were installed below the expected region of uptake by living moss tissue, making direct moss uptake less likely to influence *in situ* PO_4^{3-} -P supply to resins.

Few studies have investigated the indirect effects of non-*Sphagnum* mosses on nutrient availability—particularly phosphorus availability—through geochemical, soil microbial, or other processes. At an arctic site, Gornall et al. (2007) demonstrated that thinner moss layers resulted in warmer, wetter soils with greater microbial biomass and greater inorganic nitrogen availability than did thicker moss layers. Along an arctic-alpine altitudinal gradient, microbial activity increased with greater bryophyte and shrub cover, potentially in response to greater carbon availability (Loeffler et al. 2008). In the New Jersey pine barrens, organic matter accumulation and production of ammonium were higher in soils underlying mosses than for bare soils or lichens, while microbial respiration and nitrification were reduced under mosses (Sedia and Ehrenfeld 2005). I was unable to find work investigating the indirect effects of mosses on phosphorus cycling, specifically; however, studies of moss influences on nitrogen cycling suggest that mosses can affect soil nutrient availability by mediating

soil surface conditions, particularly with regard to microbial habitat. Demonstrated relationships between redox processes and phosphorus dynamics in rich fens at a site scale (Bailey 2006, Boomer and Bedford 2008a, b) suggest that moss effects on redox potential in particular could play a significant role.

I explored three primary indirect pathways through which the presence of mosses could influence plant-available phosphorus (inorganic phosphate) in shallow soils: through effects on (1) pH, (2) redox conditions, or (3) microbial activity in the soil. I used AEM resin strips as indicators of plant-available phosphorus in order to assess phosphate availability in the shallow, localized region most subject to moss influence, while disturbing the vegetation and soils as little as possible. It must be kept in mind, however, that *in situ* supply of phosphate to AEM resins represents an integrated index of phosphate availability in the presence of vascular plant uptake, microbial uptake, and geochemical effects on soil phosphorus pools through redox- or pH-dependent processes. Because AEM resins compete for phosphate with these other forms of uptake, total phosphate supply to resins may be low if phosphorus demand overall is high; lower phosphate underlying mosses therefore could indicate either lower phosphate availability to plants or higher phosphate demand (and successful competition with resins) where mosses occurred. Determining the relative importance of each phosphorus flux to total phosphorus availability, in order to develop a comprehensive model of phosphorus movement through rich fen soils, would require further study using an isotopic tracer (e.g., Kellogg and Bridgham 2003).

pH. As discussed above, rich fen mosses had no measurable effect on pH under field conditions. If the high cation exchange capacity of rich fen mosses enabled them to acidify their local environment, they might help make phosphate available that otherwise would be bound to calcium, similar to acid-mobilization of

phosphorus by the roots of some vascular plant species (Vance et al. 2003). In contrast, if fen mosses were to increase localized pH as shown in one laboratory experiment (Kooijman and Bakker 1994), phosphate instead might become less available. The lack of any moss effect on pH in this study suggests that rich fen mosses did not influence phosphorus availability from pH-dependent calcium-bound pools. These results corresponded to findings by Boomer and Bedford (2008a) that contrary to expectations, calcium mineral dynamics did not control site-level fen phosphorus availability.

Redox conditions. Boomer and Bedford (2008a) found that redox processes rather than calcium mineral dynamics determined phosphorus availability across fen sites, which suggested that moss effects on redox potential also might influence phosphorus availability in shallow soils. Given that the presence of mosses resulted in more oxidized soil conditions and lower concentrations of Fe^{2+} (Figure 1-1), mosses might indirectly influence phosphorus availability through increased binding of phosphate to oxidized iron (Fe^{3+}), thereby decreasing availability to plants (Smolders et al. 2006). (In contrast, under conditions where Fe^{3+} instead was reduced to Fe^{2+} , phosphate could be released from iron oxide compounds and again become available). The lower supply of phosphate to AEM resins underlying mosses than where mosses were removed suggests that moss effects on iron oxidation-reduction reactions may have influenced phosphate availability in the soil.

Microbial activity. Mosses influenced the soil biological community as well as physicochemical conditions in surface soils: microbial respiration rates and microbial biomass carbon were greater where mosses occurred than where they had been removed (Figure 1-6), suggesting that the soil microbial community could be affecting phosphorus dynamics underlying mosses. By altering factors such as soil moisture, temperature, or carbon availability, mosses could influence microbial habitat

conditions, which in turn could stimulate microbial mineralization or immobilization of phosphorus. For example, soil microbial activity tends to increase at warmer temperatures, whereas mosses have been shown to insulate the soil surface (Sveinbjornsson and Oechel 1992, Beringer et al. 2001), thereby reducing or mediating soil surface temperatures and even delaying soil thaw a full month at one arctic site (Gornall et al. 2007). In a rich fen in Michigan, mosses reduced temperature extremes without affecting average soil temperatures (Crowley and Bedford, unpublished data), which presumably would depress microbial activity or leave it unchanged. Given that microbial respiration and biomass both were greater underlying mosses, moss effects on temperature in these temperate New York fens likely were not an important regulator of microbial activity.

In wetlands, microbial activity typically is thought to increase as conditions become drier or more oxidized (reviewed in Craft 2001, Laiho 2006), suggesting that more oxidized conditions observed underlying mosses could improve conditions for microbial growth. This would contrast with upland or frozen tundra habitats, where greater moisture may stimulate microbial activity; for example, Gornall et al. (2007) suggested that wetter soils underlying thinner moss layers contributed to greater microbial activity in arctic soils. In these New York fens, soils already were saturated, and water depths generally were within 2-3 cm of the peat surface (Figure 1-2), suggesting that moisture would not limit microbial growth. Instead, more oxidized conditions underlying mosses may have promoted microbial and other biological activity, potentially increasing both phosphorus mineralization and demand for phosphorus by more active microbial communities and more vigorous vascular plants. Thus, more oxidized soil conditions where mosses occur could influence phosphorus cycling biologically as well as geochemically.

Finally, mosses have the potential to promote microbial activity by supplying labile carbon (Sedia and Ehrenfeld 2005, Loeffler et al. 2008), which frequently is considered to limit microbial growth. Moss tissues can be "leaky" (Bates and Bakken 1998), particularly when mosses are subjected to alternating wetting and drying cycles that can occur during summer months. Additionally, root biomass was greater underlying mosses than where mosses were absent (Chapter 2), potentially increasing root exudation of labile carbon compounds. A pulse of microbial growth in response to "leaked" carbon or root exudates may have increased phosphorus mineralization and the overall rate of phosphorus cycling, helping to support the high demand for phosphorus promoted by more oxidized soil conditions underlying mosses.

To explore the relative contributions of redox and microbial processes to lower levels of phosphate (or higher phosphorus demand) measured underlying mosses, I examined the extent to which $\text{PO}_4^{3-}\text{-P}$ supplied to AEM resins correlated with indicators of redox potential or microbial biomass in surface soils. Across sites and sampling times, levels of $\text{PO}_4^{3-}\text{-P}$ were negatively correlated both with indicators of higher redox potential (more oxidizing conditions) and with greater microbial biomass (Table 1-2). This analysis implies relevance for both of these indirect, interacting pathways by which rich fen mosses may influence phosphate availability in shallow soils.

Effects of mosses on phosphorus acquisition and uptake

Greater redox potential in shallow soils where mosses occurred corresponded to lower phosphate supply to AEM resins, as initially hypothesized. I next asked whether these lower phosphate levels were indicative of lower phosphorus availability to plants or of greater phosphorus demand overall—*i.e.*, did lower phosphate levels subsequently reduce microbial and plant phosphorus uptake, and therefore stimulate

alternative pathways for phosphorus acquisition, as expected if phosphorus limited growth? Greater soil phosphatase activity, root phosphatase activity for the two forb species tested, and root colonization by AMF underlying mosses (Figure 1-7, 1-8, 1-9) indicated that microbes and vascular plants did use alternative mechanisms for obtaining phosphorus from less available soil pools (*e.g.*, organic, possibly mineral-bound). These results matched those of a fen field study and greenhouse experiment where Butler (2005) demonstrated greater AMF colonization of *Solidago patula* in moss-covered areas and under conditions of decreasing phosphorus availability. Additionally, phosphatase enzyme activity often increases where levels of inorganic phosphate are low (Wright and Reddy 2001, Newman et al. 2003, Rejmankova and Sirova 2007), enabling access to phosphorus from organic sources. The lack of a difference in abundance of monesters and diesters (the most abundant forms of organic phosphorus in fen soils) in moss control vs. moss removal plots after two growing seasons (Figure 1-5) suggested that increases in phosphatase enzyme activity were more likely to derive from lower availability of inorganic phosphate underlying mosses than from differences in organic phosphorus substrates.

While increased use of alternative phosphorus acquisition mechanisms frequently indicates greater phosphorus limitation, patterns of forb and microbial phosphorus uptake did not support such an interpretation in this case. Forb tissue phosphorus concentrations and microbial phosphorus both were greater where mosses occurred than where they had been removed (Figure 1-10, 1-11), which instead would imply greater phosphorus availability and non-competitive phosphorus uptake from moss-covered soils. Greater colonization by AMF and greater phosphatase enzyme activity thus may have been stimulated by environmental factors other than lower available phosphorus, the most likely again being redox potential. More oxidized soils underlying mosses could improve growth conditions for plant roots as well as free

microbes in these saturated soils; this could enable more effective AMF root colonization and increased activation of phosphatase enzymes both by more vigorous plants and by a more active soil microbial community. Flooding may impair plant growth (Jackson and Ricard 2003, van Bodegom et al. 2008), nutrient uptake (Schat 1984), and AMF root colonization, although many fen forb species show AMF colonization in both saturated and drier soils (Weishampel 2005, Weishampel and Bedford 2006). Moreover, AMF colonization may interact with water level, such that wetland plant growth is promoted by AMF when the water table is low but inhibited by AMF when the water table is high (Weishampel 2005, Wolfe et al. 2006). Thus, the interaction between greater redox potential and greater AMF colonization in the presence of mosses may have increased plant phosphorus uptake where mosses occurred. Conversely, root growth, AMF colonization, and therefore phosphorus uptake may have been inhibited by more reducing conditions where mosses were removed, either through low oxygen availability or through iron toxicity where concentrations of Fe^{2+} were high (Snowden and Wheeler 1993, 1995).

Greater microbial and forb tissue phosphorus concentrations where mosses occurred implied that lower phosphate supply to AEM resins resulted from higher phosphorus demand underlying mosses rather than lower phosphorus availability to plants. Greater rates of microbial respiration and higher microbial biomass underlying mosses (Figure 1-6), possibly stimulated by labile carbon inputs from mosses or increased root exudation (Chapter 2), could support faster phosphorus cycling leading to greater phosphate availability in moss-covered soils. Concurrently, in more oxidized soils underlying mosses, increased phosphorus uptake both biologically (by microbes and vascular plants) and geochemically (through binding to Fe^{3+}) could explain the lower levels of phosphate supplied to AEM resins. Further work using

isotopic tracers for phosphorus or carbon would be needed to confirm these mechanisms.

Summary and conclusions

Overall, the presence of mosses clearly influenced phosphorus cycling in shallow fen soils. Most significantly, microbial phosphorus and forb tissue phosphorus concentrations were greater where mosses occurred than where they had been removed, suggesting greater phosphorus uptake from moss-covered soils. This was true even under conditions of higher phosphorus demand via plant uptake, microbial uptake, and possibly binding of phosphorus to Fe^{3+} in more oxidized soils underlying mosses.

I initially hypothesized that more oxidized conditions underlying mosses would result in lower phosphorus availability, leading to lower plant tissue phosphorus concentrations and therefore activation of alternative phosphorus acquisition mechanisms (*e.g.*, phosphatase enzymes, AMF root colonization). More oxidized conditions underlying mosses did in fact correspond to lower phosphate supply to AEM resins, greater root and soil phosphatase activity, and greater AMF colonization. Simultaneously greater microbial phosphorus and forb tissue phosphorus concentrations where mosses occurred, however, suggested that more oxidized soil conditions underlying mosses had multiple interrelated effects, stimulating: (1) greater phosphate supply through increased microbial mineralization, possibly promoted also by greater labile carbon availability; (2) greater phosphorus demand via microbial uptake, plant uptake, and geochemical iron-phosphorus reactions; and (3) greater phosphorus acquisition through improved plant vigor and greater AMF colonization. Moss effects on redox conditions in shallow soils thus may improve forb phosphorus uptake in a phosphorus-limited environment.

These biogeochemical effects of mosses have important implications for plant species composition and diversity in rich fens. Many of the forb species that comprise a high proportion of fen diversity are shallowly rooted, are therefore subject to moss influences, and are more abundant in areas where mosses occur (Chapter 3). Moss effects on redox conditions and phosphorus cycling in rich fens may be particularly critical to these species, which thus may be most successful where mosses cover the peat surface. This would suggest an important role for mosses in maintaining the high diversity and unique species composition of rich fens.

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CHAPTER 2
MOSES PROMOTE VASCULAR PLANT ROOT GROWTH IN THREE
RICH FENS IN CENTRAL NEW YORK

ABSTRACT

Rich fens are ideal systems for exploring plant root responses to environmental constraints such as phosphorus limitation and soil saturation, both of which were influenced in shallow soils by mosses covering the peat surface. I examined rich fen rooting patterns through a field survey of existing root distribution in moss-covered and naturally moss-free soils, and through a moss removal experiment investigating moss effects on root biomass, morphology, and topology (branching patterns) for seedlings of two forb species (*Solidago patula* and *Lycopus uniflorus*) and one sedge species (*Carex hystericina*). In both studies, the presence of mosses stimulated vascular plant root growth. Total root biomass from shallow (0-1 cm depth) soil core sections was greater by a factor of 2-3 where mosses occurred (15.4-36.8 g m⁻² across three sites) than where they naturally were absent (5.4-13.7 g m⁻²). Similarly, root length density was greater underlying mosses (23.7-57.4 vs. 12.9-32.5 cm cm⁻³). The moss removal experiment showed similar results for the two forb species tested, but not for the sedge: root and shoot biomass were more than double for *Solidago patula* test seedlings in moss control plots after the second growing season, and both *Solidago patula* and *Lycopus uniflorus* showed significantly greater root length, root surface area, and numbers of root branches where mosses occurred after one growing season. Most differences in root morphological measures where mosses were present or absent were consistent both with more oxidized soils underlying mosses and with a root foraging response to greater localized phosphorus availability. Higher above- and belowground forb seedling biomass indicative of greater plant vigor, as well as the

absence of root branching patterns typical of nutrient foraging, suggested that more oxidized soil conditions may have been the primary driver of forb root responses to the presence of mosses. In rich fens, moss influences on plant rooting may be significant in helping a high diversity of shallowly rooted forb species to succeed in a saturated and phosphorus-limited environment.

INTRODUCTION

Interactions between above- and below-ground processes play a key role in the flow of materials and energy and in the imposition or alleviation of environmental constraints (Wardle 2002 and references therein, Gutierrez and Jones 2006). Aboveground movement and use of materials (e.g., nutrients, carbon) and effects on physicochemical conditions (e.g., pH, temperature, oxygen availability) may determine productivity, nutrient cycling, and other ecosystem processes belowground (and vice versa). For example, conditions aboveground may have critical effects on the growth and turnover of roots, which influence belowground functioning through significant contributions to biomass (Jackson et al. 1996), nutrient concentrations (Craine et al. 2003), and soil respiration and organic matter pools (Gaudinski et al. 2000). While critical, however, these kinds of interactions may be indirect and often difficult to explore.

Rich fens, alkaline peatlands fed primarily by groundwater, are ideal systems for exploring such linkages because of their strong physicochemical constraints combined with a unique boundary condition at the soil surface that directly influences the biogeochemistry of shallow soils. In rich fens, water levels are consistently at or near the soil surface and nutrients (particularly phosphorus or a combination of nitrogen and phosphorus) typically limit plant growth (Bedford et al. 1999, Bedford and Godwin 2003, Boomer and Bedford 2008), forcing plant species to contend with

the combined stresses of soil saturation and nutrient limitation. Additionally, mosses cover much of the peat surface directly at the aboveground-belowground boundary, where they may influence both of these conditions (Chapter 1). Mosses have been shown to affect temperature, moisture, nutrient availability, organic matter accumulation, and microbial activity in wetlands and other systems (Vitt 2000, Turetsky 2003, Sedia and Ehrenfeld 2005, 2006, Cornelissen et al. 2007, Gornall et al. 2007), and they affect redox conditions and phosphorus cycling in rich fens, specifically (Chapter 1). Mosses also are known to facilitate or impede plant growth aboveground (e.g., Svensson 1995, Malmer et al. 2003); however, little is known about how moss influences on physicochemical conditions may affect plant activity belowground.

Plant roots may respond to constraints imposed either by nutrient limitation or soil saturation in related ways. Where nutrient concentrations are low overall, root biomass may be greater to enable sufficient nutrient uptake to support aboveground growth (Hutchings and John 2003); for example, in a Louisiana salt marsh, Darby and Turner (2008) found live root biomass to decrease 40-60% with phosphorus additions. If exposed to localized areas of greater nutrient availability within a nutrient-limited environment, however, roots may proliferate to take advantage of the nutrient patches at this scale (Hutchings and John 2003, Hodge 2004, 2006 and references therein). In wetland habitats, roots must contend with soil saturation as well as nutrient constraints: saturated or flooded soils may suppress root growth (e.g., Kotowski et al. 2001, Kercher and Zedler 2004, Luo and Xie 2009) even across slight differences in water level, particularly when water levels are at or above the soil surface (Fraser and Karnezis 2005).

Interacting effects between nutrient limitation and soil saturation on belowground plant growth are less well understood. Jansen (2005, 2007) determined

that floodplain wetland plant species were able both to respond selectively to soil nutrient heterogeneity (to a greater extent even than species of drier habitats) and to grow successfully under flooded conditions, but only one of four species tested maintained equivalent root biomass during flooding. Addressing related questions, Bouma et al. (2001b) demonstrated for salt marsh forb species that root branching patterns indicative of root foraging were typical of species of higher elevations, potentially to enable greater nutrient uptake where competition was more intense. In contrast, root traits and branching patterns adapted to a lower nutrient and wetter environment were typical for species of lower elevations, where they may have aided root survival by reducing oxygen loss or susceptibility to phytotoxins (Bouma et al. 2001b). Thus, wetland plant species clearly show plasticity in root morphology and topology (root branching patterns) in response to nutrient heterogeneity and changes in water level, but less is known about interactions between these variables for wetland plant species in a field setting.

The position of mosses at the soil surface in rich fens provides an opportunity to explore these kinds of interactions. In New York fens, where mosses occurred, surface soils were more oxidized and tissue phosphorus concentrations for shallowly rooted forb species were greater, suggesting greater phosphorus availability to plants from moss-covered soils (Chapter 1). This work also suggested that more oxidized soils were a key driver of greater microbial activity, higher root phosphatase enzyme activity, and greater root colonization by arbuscular mycorrhizal fungi (AMF) underlying mosses, thus promoting increased phosphorus supply and acquisition (Chapter 1). Given the high sensitivity of diagnostic fen plant species to water level (Kercher and Zedler 2004), more oxidized conditions underlying mosses also might alleviate constraints on physical root growth, thereby stimulating root proliferation near the soil surface. With regard to greater phosphorus availability underlying

mosses, roots might respond in one of two ways. Greater access to phosphorus from moss-covered soils could reduce the need for root growth, therefore resulting in lower root biomass overall. Alternatively, greater localized phosphorus availability in the narrow zone immediately underlying mosses might stimulate a root foraging response, given that phosphorus frequently limits fen plant growth (Bedford et al. 1999, Bedford and Godwin 2003), and wetland plant species have shown high plasticity in response to nutrient heterogeneity (Jansen et al. 2005, Jansen 2007). Thus, differences in plant rooting patterns in areas where mosses are present or absent could clarify the relative importance of soil redox conditions and phosphorus availability in influencing the growth of shallowly rooted fen plants.

The effects of fen mosses on redox conditions and phosphorus availability may be particularly important to the forb species that comprise much of the high plant diversity characteristic of rich fens (Bedford and Godwin 2003). The vascular plant species composition of rich fens typically consists of a dominant matrix of sedge species, with a diverse community of forb species interspersed among them. These shallowly rooted forbs may be anchored in the upper layer of peat where the effects of mosses on surface soil conditions are prominent. Additionally, among herbaceous plants, forbs have been more likely than grasses (and likely other graminoids) to show a plastic root response to changes in environmental conditions and to proliferate roots into localized areas of higher nutrient concentration (Fitter and Stickland 1992, Taub and Goldberg 1996, Bouma et al. 2001a, Bouma et al. 2001b). In contrast, for a fen sedge species typical of low-phosphorus conditions, lack of such plasticity was demonstrated both for root biomass and some morphological characteristics, although a sedge species of more nutrient-rich conditions was more responsive (Perez-Corona and Verhoeven 1996, 1999). Based on these trends, I would expect a differential root

response between rich fen forb and sedge species to the presence or absence of mosses.

I used a moss removal experiment to explore the effects of mosses on root morphology and topology for seedlings of two fen forb species (*Solidago patula* Muhl. ex Willd. (roundleaf goldenrod) and *Lycopus uniflorus* Michx. (northern bugleweed)) and one sedge species (*Carex hystericina* Muhl. ex Willd. (bottlebrush sedge)). I also characterized root abundance and distribution with depth for the existing, sedge-dominated fen vegetation in moss-covered vs. naturally moss-free areas. To explore trade-offs in plant root response to differences in soil redox conditions vs. phosphorus availability, I asked the following questions:

1. Is root proliferation greater where mosses occur or where they are absent? Root proliferation where mosses occur could be a response to more oxidized soils, a localized foraging response to greater phosphorus availability immediately underlying mosses, or a combination of the two. Alternatively, root proliferation where mosses are absent could be a response to lower availability of phosphorus to plants overall.
2. Do fen forb species demonstrate greater morphological root plasticity than does a fen sedge species?
3. How are root responses to the presence or absence of mosses reflected in plant uptake of phosphorus, which is often a limiting nutrient in these wetlands?

METHODS

Study sites

I performed a field survey of existing vascular plant root distribution with depth at three rich fens and assessed seedling root response to moss removal at two fens in central New York. Larry's Fen (LF), Clark Street Extension Fen (CSE), Fish

Fen (FF), and Belle School Fen (BSF) are in the vicinity of Cortland, NY (42.6°N, 76.2°W). LF is a sloping fen fed by hillside seepage, with vegetation dominated by a matrix of *Carex* L. species, *Dasiphora floribunda* (Pursh) Raf. (shrubby cinquefoil), mixed forbs, and the moss species *Campylium stellatum* (Hedw.) C. Jens. CSE has a similar plant species composition but slopes more gently and is dominated by *Campylium stellatum* and *Sphagnum warnstorffii* Russ. in the moss layer. BSF is a sloping fen dominated by *Typha latifolia* L. (broadleaf cattail), *Solidago patula* Muhl. ex Willd. (roundleaf goldenrod), *Packera aurea* (L.) A. & D. Löve (golden ragwort), additional mixed forbs, and diverse moss species including *Campylium stellatum*, *Brachythecium* sp. Schimp., *Sphagnum warnstorffii*, and others. FF is a sloping depressional fen dominated by *Typha angustifolia* L. (narrowleaf cattail), diverse forbs, and the moss species *Calliergonella cuspidata* (Hedw.) Loeske and *Campylium stellatum* plus a number of less frequent mosses. The moss layers in the four sites (which include a minimal abundance of liverwort species) ranged in depth from approximately 3-5 cm and ranged in coverage from nearly continuous (85-95% cover) to highly patchy (20-90% cover).

LF, CSE, and BSF occur within predominantly agricultural watersheds, and CSE has a known history of past cattle grazing. While FF is surrounded mainly by steeply sloping forest and is located within a natural area, it served as a millpond during the late 1800s and has accumulated a minimum of 25-35 cm of new peat since abandonment. At all three sites, water levels remain within 5-10 cm of the peat surface throughout the growing season.

Field survey

To assess vascular plant root distribution for the existing, sedge-dominated vegetation where mosses occurred and naturally were absent, I established five sets of

paired 10x10-cm plots in moss-covered and adjacent moss-free areas at each of three fens (LF, CSE, and BSF). Plots were paired to control for variability in rooting conditions across the sites. Within each plot, I collected a 5x5-cm soil core to a depth of 10 cm. Each core was collected by cutting the peat around a 5x5-cm surface template to depths indicated by 5x10-cm vertical templates to prevent compaction and obtain a consistent volume. Before extracting roots from the cores, I first gently removed the living moss layer where present, such that the soil core began at the peat surface for both core types. The living moss layer was defined as green tissue (ranging from 2-3 cm depth) plus firmly attached brown tissue (1-2 cm). Once mosses were removed, I sliced each core into 10 sections of 1-cm thickness.

To examine the distribution of root biomass and root length with soil depth, I extracted roots from three depth sections per core: 0-1-cm, 4-5-cm, and 8-9-cm. After weighing each core section, I washed the sample with de-ionized water in a 1-mm sieve over a 250- μ m sieve to be sure that no roots were lost. Fine roots then were separated from the remaining peat, rhizomes, and dead roots under a dissecting microscope, using a consistent definition of live roots as un-torn roots that held their structure. Nearly all live roots were less than 1 mm in diameter and no roots were greater than 2 mm in diameter.

After separating the roots from the peat, I weighed, dried at 60 degrees C, and re-weighed approximately half of each sample to estimate root biomass, and preserved the dried samples for tissue phosphorus analysis. I stored the remainder of each sample in 50% ethanol at 35 degrees C for scanning. After scanning (described below), these subsamples also were dried and weighed for calculation of specific root length (SRL; cm root per g dry root tissue) and specific root area (SRA; cm² root per g dry root tissue).

Moss removal experiment

In June 2005 at LF and FF, I established five sets of paired 0.5 x 0.5 m randomly assigned moss removal and moss control plots at each fen. Treatments within each pair included a moss control plot with no manipulation and a moss removal plot in which mosses were removed while all other plants (predominantly forbs and graminoids) were left in place. In removal plots, all living mosses were removed by hand according to the protocol used for the field survey. Treatment blocks were established in regions of each fen where the dominant moss species were "brown" mosses (typically in the family *Amblystegiaceae*), predominantly *Campylium stellatum* or *Calliergonella cuspidata*, with a minimum total moss cover of 80%. The moss removal treatment was maintained at least twice per growing season to counteract moss re-colonization.

After moss removal plots had been established, I planted 4-week-old seedlings of *Carex hystericina* and *Solidago patula* (typical fen sedge and forb species) in moss control and moss removal plots at the two sites. Because seedlings planted at FF underwent nearly complete herbivory, in 2006 I planted seedlings of *Carex hystericina*, *Solidago patula*, and *Lycopus uniflorus* (adding a second forb species) in moss control and moss removal plots and protected the seedlings with wire mesh cages. I planted 6 seedlings per species per plot in 2005 and 3 seedlings per species per plot in 2006, and I paired seedlings by height within each block. The base of each plant was positioned carefully at the peat surface in both plot types to ensure that seedling elevation would be consistent relative to the water table. Because living mosses separated cleanly from the peat, the peat surface could be identified consistently in moss control and moss removal plots, ensuring that seedling responses would be comparable between treatments.

At LF in September 2005 and at LF and FF in September 2006, I randomly selected one pair of seedlings (moss control and moss removal) per species per treatment block. I carefully extracted plants by hand, collecting the root ball with attached peat and following individual lateral roots through the soil to minimize root breakage. In the lab I separated the roots from the peat and cleaned them with deionized water. Cleaned root systems were stored in deionized water at 35 degrees C for no more than 24 hours before scanning for architectural analysis. After scanning, roots were dried at 60 degrees C, weighed to determine biomass, and preserved for tissue phosphorus analysis.

Root analysis

To determine root length and diameter distribution for root core samples, I stained roots with 0.33 g/L neutral red dye for a minimum of 24 hours, following methods modified from Bouma et al. (2000). I then rinsed the root segments with deionized water and spread them in a clear tray in a thin layer of water. I used WinRHIZO (v.2007c, Regent Instruments Inc. 2007) connected to an Epson Expression 1680 scanner with transparency lighting system to acquire a 600 dpi image and analyze it for root length, surface area, and diameter. The WinRHIZO automatic threshold settings were used for the initial scan, supplemented by higher threshold settings to capture missed roots in localized portions of the image (Regent Instruments Inc. 2007).

For intact root systems of seedlings from moss control and moss removal plots, I spread each cleaned root system in water in a transparent tray for an initial scan. For *Solidago patula*, I then separated each basal root with attached laterals from the main taproot (root nomenclature follows Gregory (2006)) and scanned them sequentially from the base of the plant so that root overlap could be minimized. To characterize

each root system, I analyzed the uppermost basal root (as the root most likely to be influenced by mosses) plus two (2005) or three (2006) additional randomly selected roots for morphology and topology. Each root first was scanned and analyzed using WinRHIZO threshold settings to identify undetected sections of the root, which then were traced as needed at a magnification of 3-4 times to create a complete image of the root (Regent Instruments Inc. 2007). A topological link analysis (analysis of branching patterns, where a link is a root section between branches) was performed for each basal root using the finalized images (Fitter 1996). I followed the same scanning and topological analysis protocol for *Carex hystericina*, but the sequential order of scanning was necessarily less precise, given that all roots originate from the same node. *Lycopus uniflorus* root systems were smaller than those of the other two species and therefore could be analyzed in their entirety.

For comparability among species, I characterized root systems by summarizing root length, surface area, average diameter, the number of root tips, external link length, and internal link length per basal or nodal root. This was done either by averaging these measures by the total number of roots analyzed (*Solidago patula* and *Carex hystericina*), or by dividing values for the total root system by the number of basal roots per plant (*Lycopus uniflorus*). In addition to root morphological characteristics, I calculated a topological index (TI) from the altitude and magnitude [TI = $\log(\text{altitude})/\log(\text{magnitude})$, where altitude is the number of links in the longest path from an exterior link (with a root tip) to the basal (initial) link, and magnitude is the total number of exterior links or root tips], either per basal or nodal root (*Solidago patula*, *Carex hystericina*) or for the full root system (*Lycopus uniflorus*) (Fitter 1996, Bouma et al. 2001b). TI was calculated in order to characterize root branching patterns as more dichotomous (lower TI) or more herringbone (higher TI) in nature (Fitter 1987, Fitter 1996).

Plant tissue chemistry

To analyze plant root and shoot tissues for phosphorus, I digested dried shoots, dried roots, apple-leaf Standard Reference Material (NIST SRM 1515), and blanks (to check for phosphorus contamination during digestion) in a heated aluminum block in 1.5 mL concentrated sulfuric acid (H_2SO_4) with hydrogen peroxide (H_2O_2) (Allen 1989, Weishampel 2005). I added 2 mL of H_2O_2 in 0.5 mL aliquots, raised the temperature to 320 degrees C over a 90-minute period, and held this temperature for 20 minutes to boil away un-reacted H_2O_2 . I then allowed the samples to cool and repeated the H_2O_2 additions if needed to achieve a colorless digest. Cooled digests were diluted to 25 mL, and samples, apple-leaf standards, and blanks were analyzed for total phosphorus through inductively coupled plasma (ICP) spectroscopy at the Cornell Nutrient Analysis Laboratory, Ithaca, NY.

Data analysis

I analyzed data from the field survey and moss removal experiment using generalized linear mixed models (proc MIXED, SAS version 9.1). For the field survey, I analyzed the effect of moss presence on each response variable (root length density, average root diameter, etc.) using block as a random factor and site, soil depth, and the presence or absence of mosses as fixed factors. Where moss presence interacted with soil depth, I tested the effect of mosses for each depth.

For the moss removal experiment, I separated the analysis into two stages, first analyzing the effect of moss removal on morphological, topological, and tissue phosphorus response variables for all three species at both sites for a single growing season. I then analyzed response variables for *Solidago patula* at LF across two growing seasons using a repeated measures analysis in which year was the repeated measure for each site-block-plot combination. (*Solidago patula* was the only species

with sufficient seedling survival to allow a two-year analysis.) Where treatment interacted with species, I tested the effect of treatment for each species. For both the field survey and the moss removal experiment, I log-transformed variables where needed to satisfy model assumptions of normality and homogeneity of variance, and I used Satterthwaite's approximation to estimate denominator degrees of freedom (Littell et al. 2006).

RESULTS

Root distribution with depth for existing vegetation

In the field survey, root biomass (mg root per cm³ soil) from shallow (0-1 cm depth) core sections taken in the existing, sedge-dominated rich fen vegetation was greater by a factor of 2-3 where mosses occurred than where they naturally were absent (Figure 2-1a). Similarly, root length density (cm root per cm³ soil) on average was 70% greater underlying mosses (Figure 2-1b). In contrast, specific root length (cm per g root) and specific root area (cm² per g root) were 20-30% lower in moss-covered than in moss-free soils (Figure 2-2), which corresponded to a greater average root diameter immediately underlying mosses (Figure 2-3). These differences were strongest in the upper 0-1 cm of soil and in most cases equilibrated by a depth of 4-5 or 8-9 cm (Figure 2-1, 2-2, and 2-3). Differences in root distribution between moss-covered and bare soils generally persisted to the greatest depth at Larry's Fen (LF).

Seedling root responses to moss removal

For paired *Solidago patula* seedlings planted at LF, root and shoot biomass were more than twice as great in moss control than in moss removal plots after two growing seasons (Figure 2-4). In contrast, *Carex hystericina* seedlings appeared to

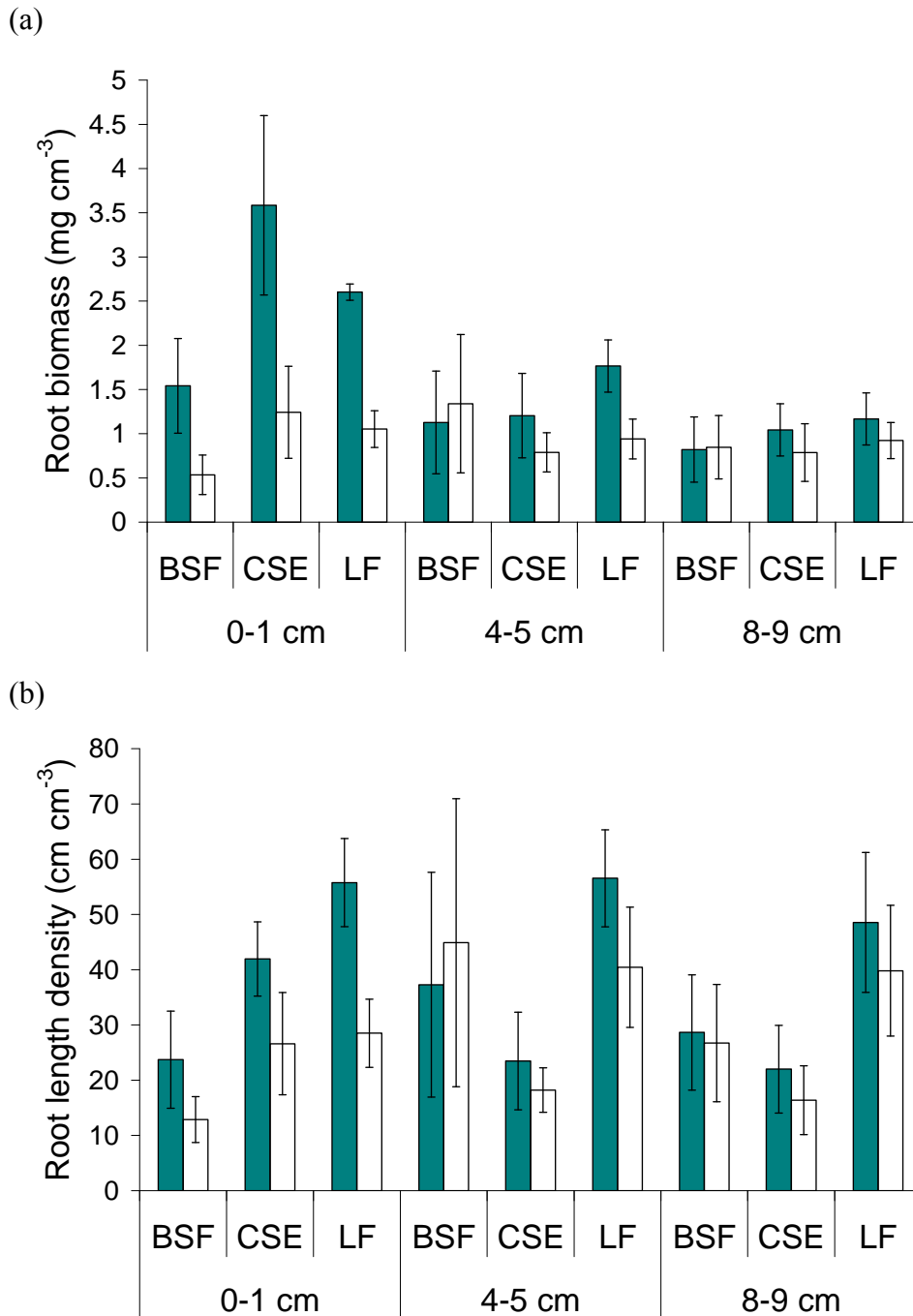


Figure 2-1. Differences in existing (a) root biomass and (b) root length density with depth between areas with mosses present (solid bars) vs. naturally absent (open bars) at three New York fens. Differences were significant at a depth of 0-1 for root biomass ($p < 0.0001$) and across the three depths for root length density (no depth x moss interaction; $p = 0.01$). BSF = Belle School Fen, CSE = Clark Street Extension Fen, and LF = Larry's Fen. Error bars \pm SE, $n = 5$.

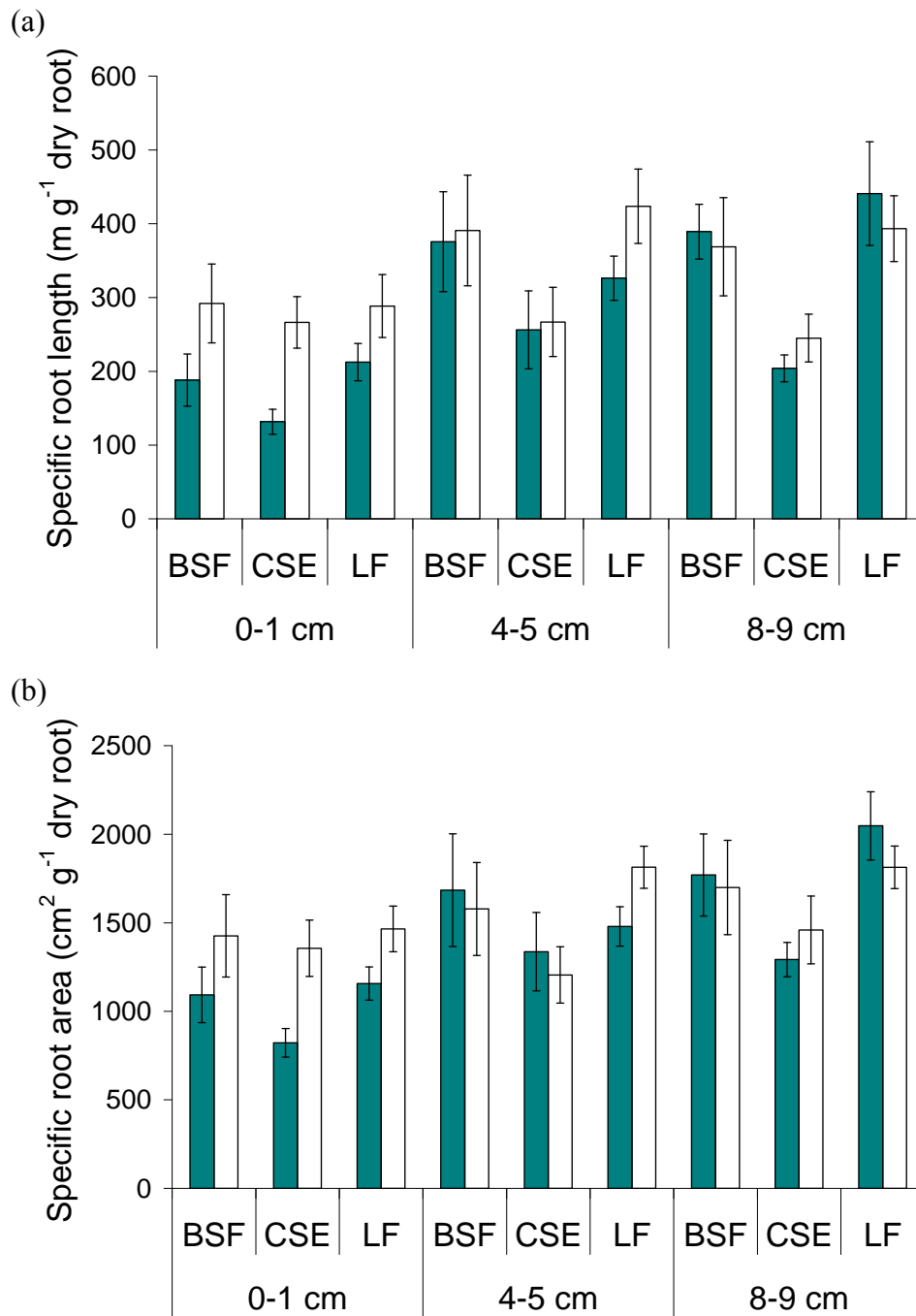


Figure 2-2. Differences in (a) specific root length and (b) specific root area with depth between areas with mosses present (solid bars) vs. naturally absent (open bars) at three New York fens. Differences were significant at a depth of 0-1 for both measures ($p < 0.01$). BSF = Belle School Fen, CSE = Clark Street Extension Fen, and LF = Larry's Fen. Error bars \pm SE, $n=5$.

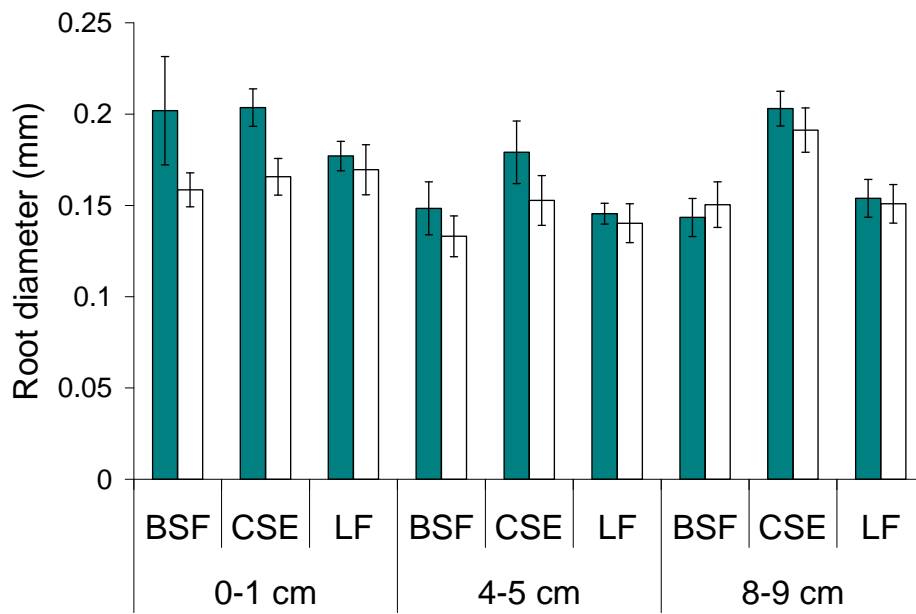


Figure 2-3. Differences ($p < 0.01$ overall) in average root diameter with depth between areas with mosses present (solid bars) vs. naturally absent (open bars) at three New York fens. BSF = Belle School Fen, CSE = Clark Street Extension Fen, and LF = Larry's Fen. Error bars \pm SE, $n=5$.

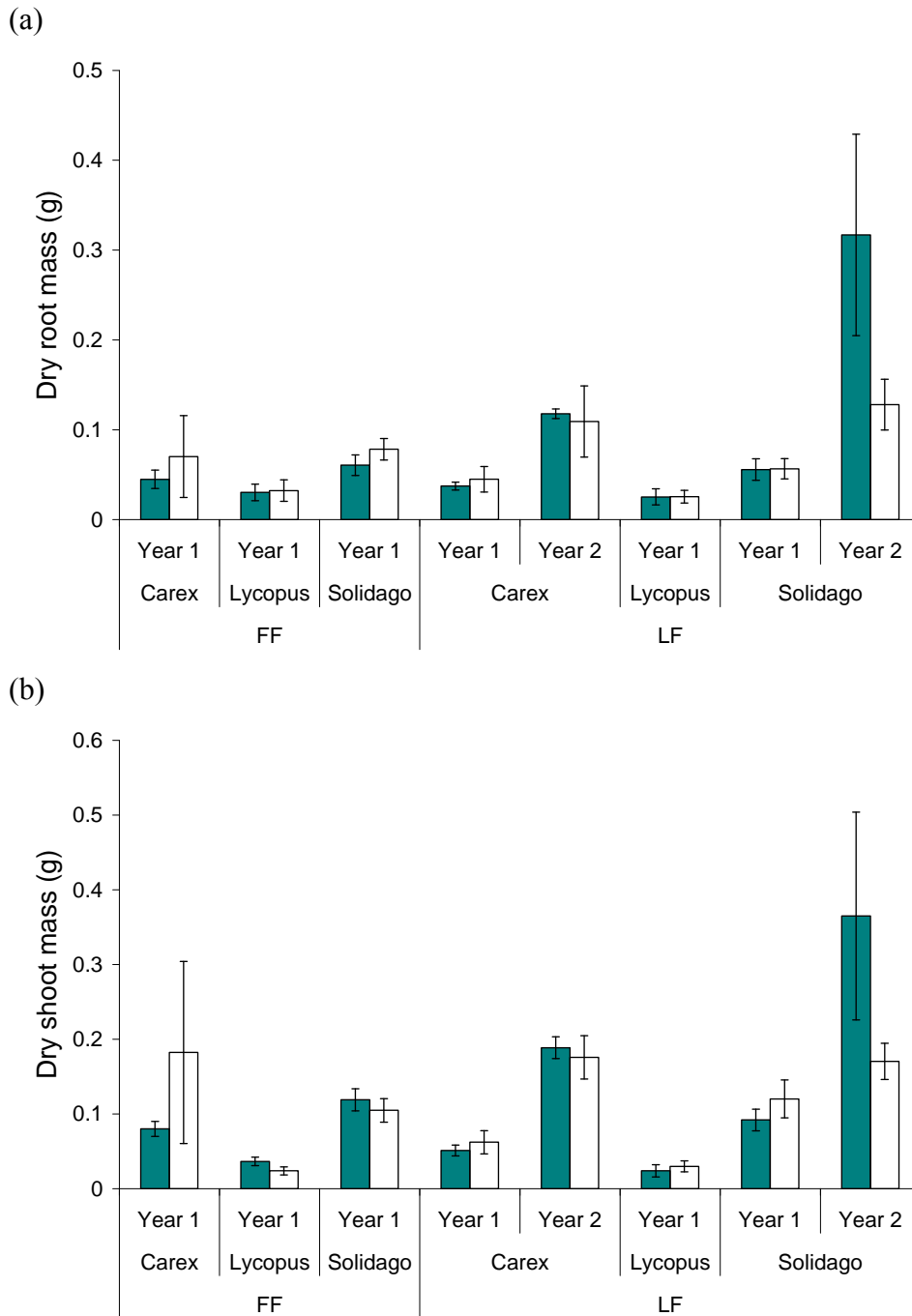


Figure 2-4. Differences in (a) root biomass and (b) shoot biomass between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina*, *Lycopus uniflorus*, and *Solidago patula* at Fish Fen (FF) and Larry's Fen (LF), harvested after one or two growing seasons (indicated as year 1 or year 2). Control plots had greater root biomass ($p < 0.01$) and marginally greater shoot biomass ($p < 0.1$) only for *Solidago patula* in year 2. Error bars \pm SE, $n = 5$.

show no difference in root or shoot biomass after two growing seasons, although the second growing season could not be examined statistically because of insufficient seedling survival. None of the three species, including *Solidago patula*, showed a biomass response to moss removal after a single growing season (Figure 2-4). Additionally, the ratio of root to shoot biomass did not differ significantly between treatments (Figure 2-5).

While a seedling biomass response to moss removal was evident only in the second growing season, root morphological characteristics for the two forb species (*Solidago patula* and *Lycopus uniflorus*) responded to moss removal by the end of the first growing season. Root length, surface area, and the number of root branches (indicated by the number of root tips) all were greater where mosses occurred than where they had been removed (Figure 2-6 and 2-7). Additionally, the length and surface area of both exterior and interior root links for the two forb species were greater in moss control than in moss removal plots (Figure 2-8). In contrast to results from the root cores, which were dominated by mature sedge roots, specific root length and specific root area for seedlings of the two forb species also were greater where mosses occurred (Figure 2-9), which corresponded to a marginally lower average root diameter ($p < 0.1$). While seedlings of the sedge species *Carex hystericina* showed no significant response to moss removal in root length, surface area, branching, specific root length, or specific root area (Figure 2-6, 2-7, 2-8, and 2-9), the direction of response was opposite that of the forb species, suggesting the possibility of lower root growth for this sedge species where mosses occurred than where they had been removed.

Differences in root length, surface area, and branching between moss control and moss removal treatments were not reflected in the topological index (TI) for the test seedlings (Figure 2-10). TI ranged from 0.6-0.9 and did not differ significantly

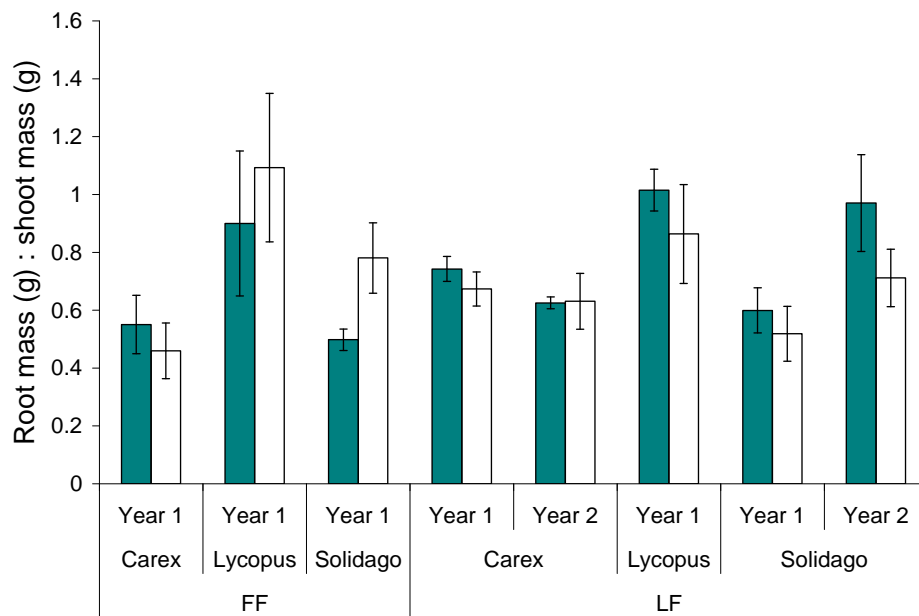


Figure 2-5. Differences in the ratio of root to shoot biomass (n.s.) between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina*, *Lycopus uniflorus*, and *Solidago patula* at Fish Fen (FF) and Larry's Fen (LF), harvested after one or two growing seasons (indicated as year 1 or year 2). Error bars +/- SE, n=5.

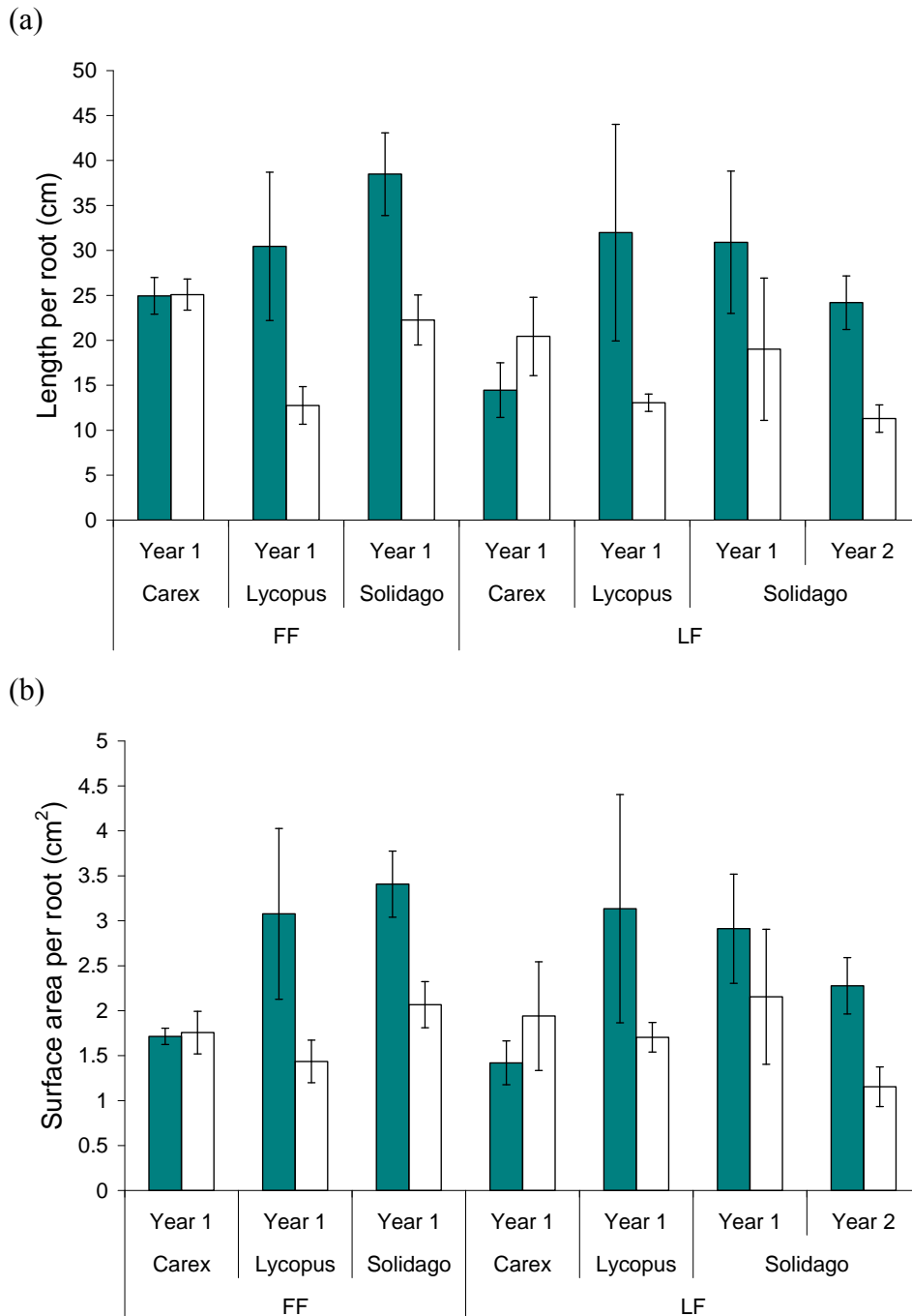


Figure 2-6. Differences in (a) root length and (b) surface area per nodal or basal root between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina* (n.s./n.s.), *Lycopus uniflorus* ($p < 0.001/p < 0.01$), and *Solidago patula* ($p < 0.01/p < 0.05$, across sites for year 1 and across years for LF). Seedlings were planted in treatment blocks at Fish Fen (FF) and Larry's Fen (LF) and harvested after one or two growing seasons (indicated as year 1 or year 2). Error bars \pm SE, $n=5$.

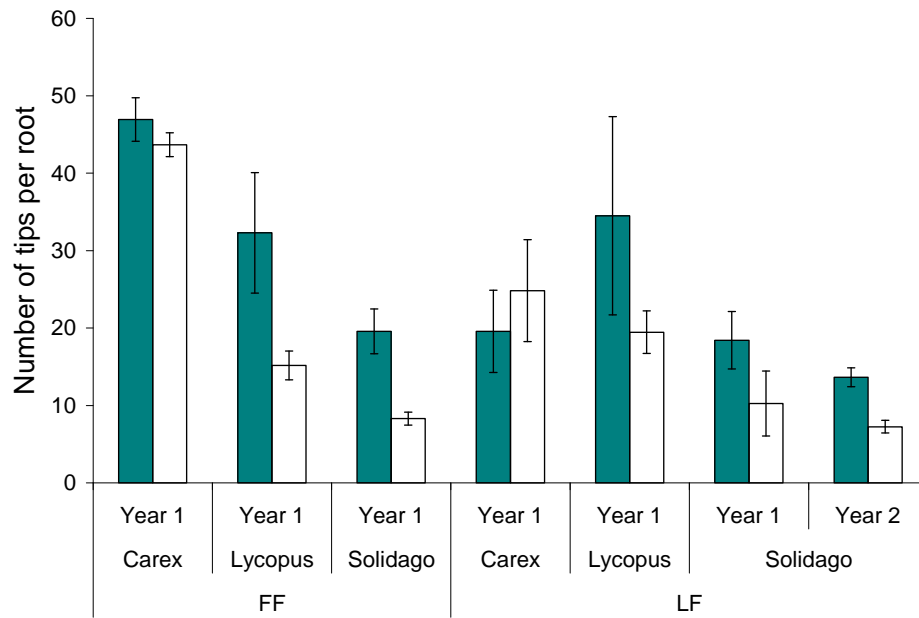


Figure 2-7. Differences in the number of root tips (magnitude) per nodal or basal root between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina* (n.s.), *Lycopus uniflorus* ($p < 0.05$), and *Solidago patula* ($p < 0.01$, across sites and years). Seedlings were planted in treatment blocks at Fish Fen (FF) and Larry's Fen (LF) and harvested after one or two growing seasons (indicated as year 1 or year 2). Error bars \pm SE, $n=5$.

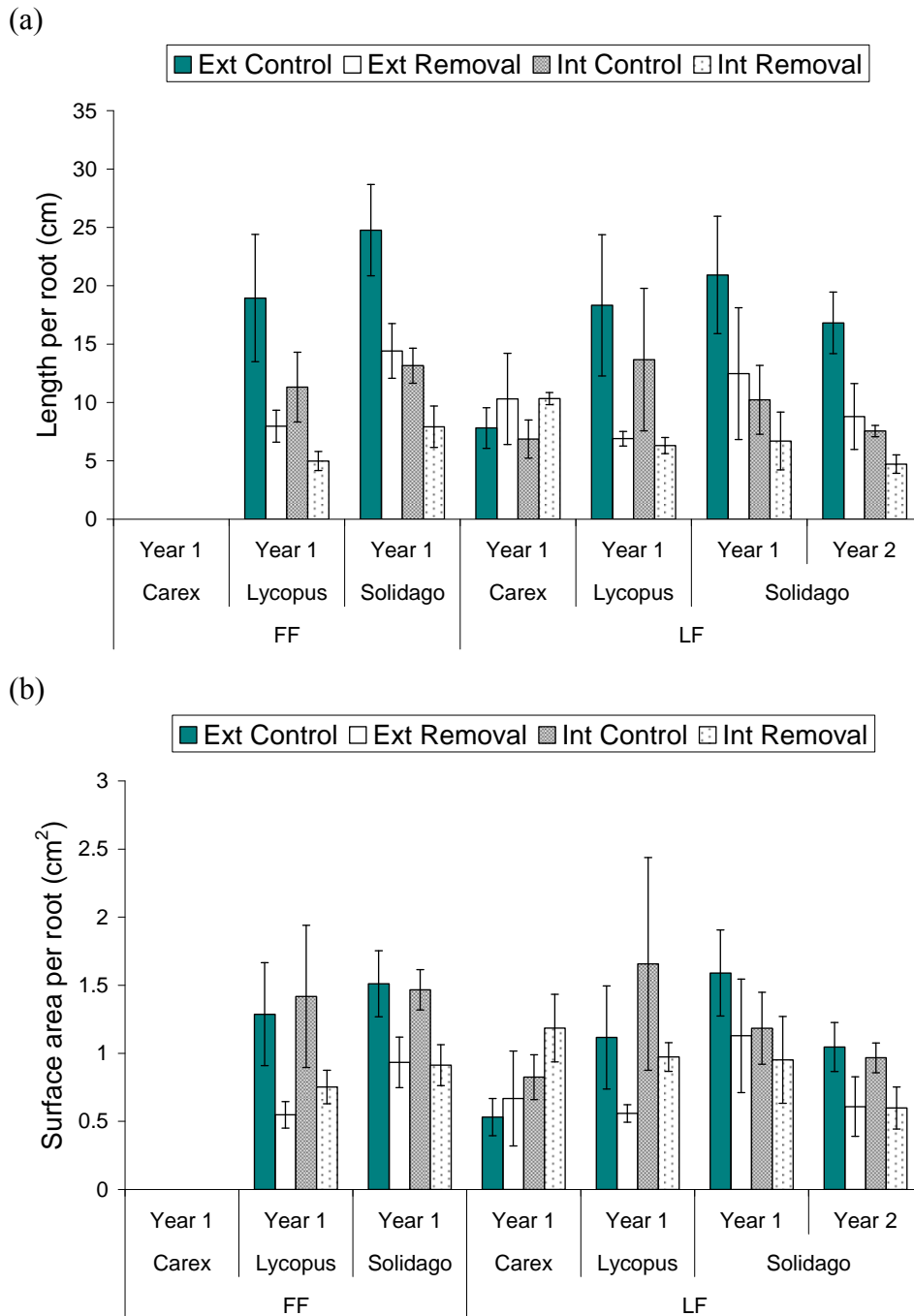


Figure 2-8. Differences in (a) root length and (b) surface area for exterior and interior root links in moss control vs. moss removal plots for test seedlings of *Carex hystericina* (n.s./n.s.), *Lycopus uniflorus* ($p < 0.001/p < 0.001$), and *Solidago patula* ($p < 0.001/p < 0.01$, across sites for year 1 and across years for LF). Seedlings were harvested at Fish Fen (FF) and Larry's Fen (LF) after one or two growing seasons (indicated as year 1 or year 2). Root systems for *Carex hystericina* from FF were too complex to allow topological analysis of scanned images. Error bars \pm SE, $n=5$.

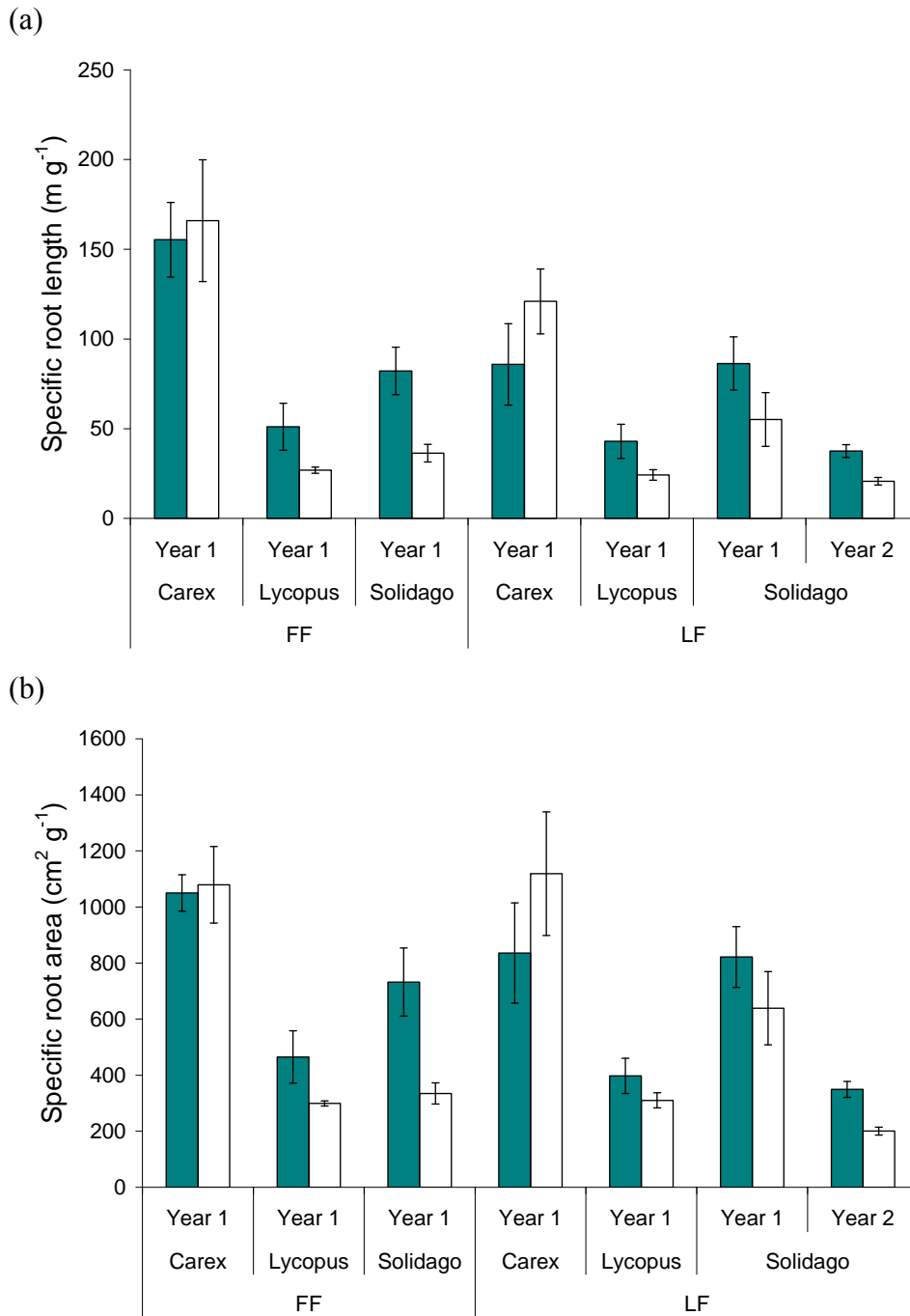


Figure 2-9. Differences in (a) specific root length and (b) specific root area between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina* (n.s./n.s.), *Lycopus uniflorus* ($p < 0.01/p < 0.05$), and *Solidago patula* ($p < 0.01/p < 0.01$, across sites for year 1 and across years for LF). Seedlings were planted in treatment blocks at Fish Fen (FF) and Larry's Fen (LF) and harvested after one or two growing seasons (indicated as year 1 or year 2). Error bars +/- SE, $n = 5$.

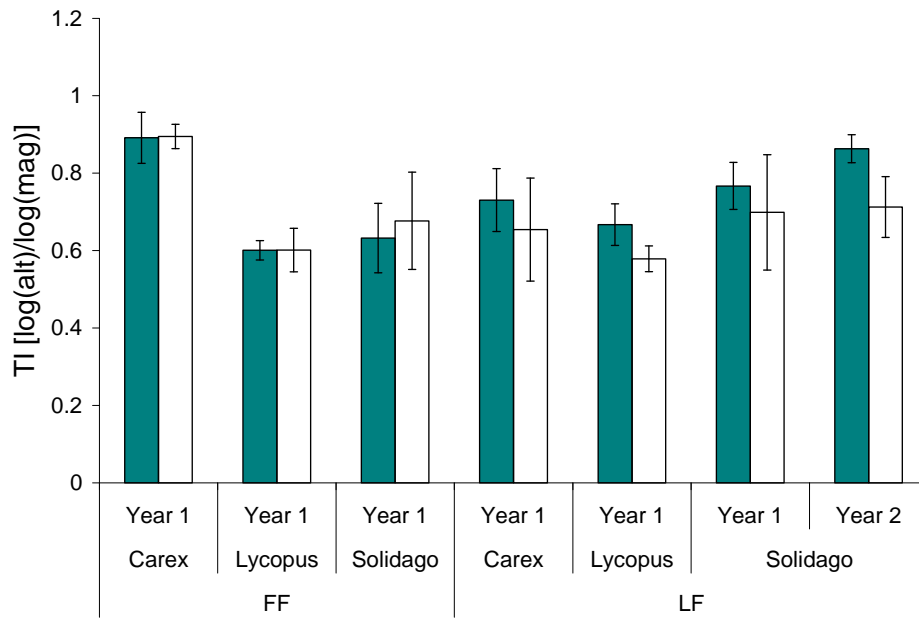


Figure 2-10. Topological index (TI = log(altitude)/log(magnitude)) per nodal or basal root for test seedlings of *Carex hystericina* and *Solidago patula*, and per plant for seedlings of *Lycopus uniflorus*. Seedlings were planted in moss control (solid bars) and moss removal plots (open bars) at Fish Fen (FF) and Larry's Fen (LF) and harvested after one or two growing seasons (indicated as year 1 or year 2). No significant differences in TI were observed. Error bars +/- SE, n=5.

between treatments, although a trend was suggested toward higher TI values at LF where mosses occurred than where they had been removed (Figure 2-10). TI was greatest for *Carex hystericina* and lowest for *Lycopus uniflorus*, but values varied within species and within sites, suggesting no clear trend in herringbone vs. dichotomous branching patterns for these species or locations.

Effects of mosses on plant phosphorus uptake

Between areas with mosses present vs. naturally absent, root tissue phosphorus concentrations representing existing, sedge-dominated vegetation did not differ significantly (Figure 2-11). For test seedlings planted in moss control and moss removal plots at Fish Fen (FF) and LF, however, shoot tissue phosphorus concentrations for the two forb species were greater (*Lycopus uniflorus*) or marginally greater (*Solidago patula*) where mosses occurred (Figure 2-12a). Root tissue phosphorus concentrations for the two forb species also generally were greater where mosses occurred, except for *Solidago patula* seedlings planted at LF, for which no treatment effect was evident (Figure 2-12b). In contrast to the two forb species, tissue phosphorus concentrations for the sedge species *Carex hystericina* did not respond to mosses, although the direction of the trend at LF was toward lower shoot and root tissue phosphorus concentrations where mosses occurred than where they had been removed (Figure 2-12). The lack of a tissue phosphorus response for *Carex hystericina* seedlings was consistent with the lack of response in the core samples of existing vegetation, which would be dominated by a combination of young and mature roots of several sedge species.

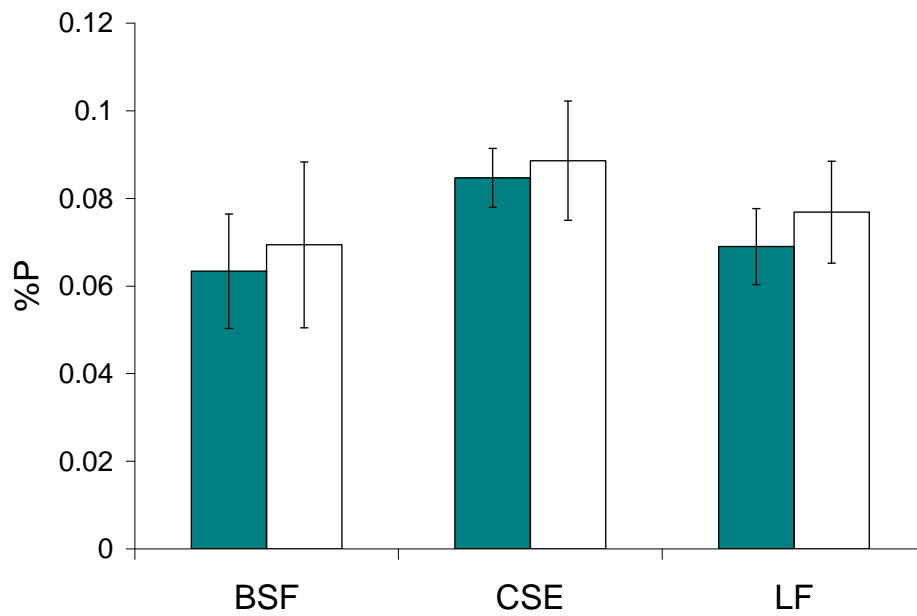


Figure 2-11. Differences in root tissue phosphorus concentrations from a depth of 0-1 cm (n.s.) between areas with mosses present (solid bars) or naturally absent (open bars). Root samples are from soil core sections from three New York fens: Belle School Fen (BSF), Clark Street Extension Fen (CSE), and Larry's Fen (LF). Error bars +/- SE, n=5.

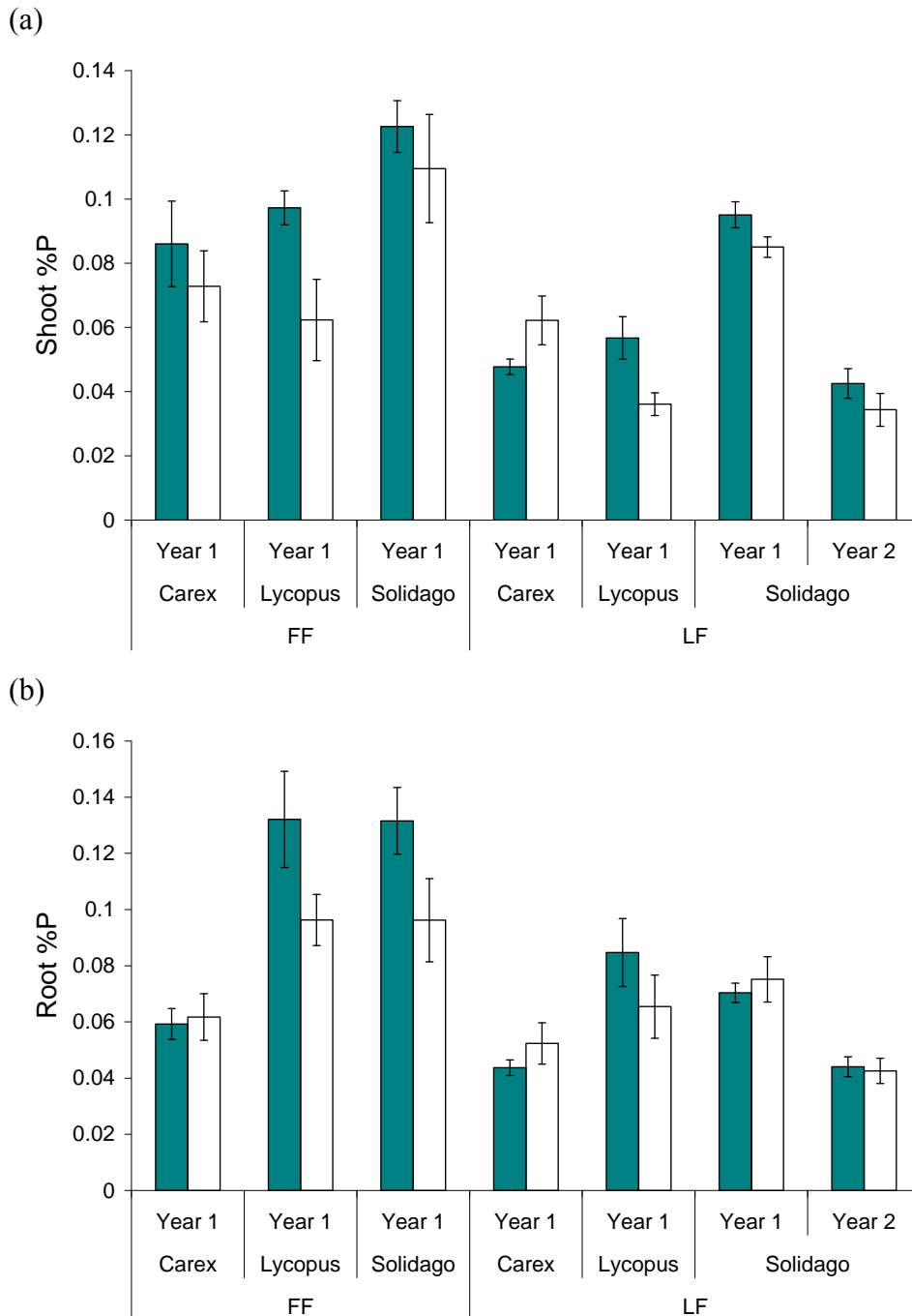


Figure 2-12. Differences in (a) shoot and (b) root tissue phosphorus concentrations between moss control (solid bars) and moss removal plots (open bars) for test seedlings of *Carex hystericina* (n.s./n.s.), *Lycopus uniflorus* ($p < 0.01/p < 0.01$), and *Solidago patula* ($p = 0.1/p = 0.1$ across sites; $p < 0.05$ for shoot %P at LF across years). Seedlings were planted in treatment blocks at Fish Fen (FF) and Larry's Fen (LF) and harvested after one or two growing seasons (indicated as year 1 or year 2). Error bars \pm SE, $n = 5$.

DISCUSSION

Roots proliferated where mosses occurred

Both for existing vegetation in natural field conditions and for seedlings responding to experimental manipulation, the presence of mosses stimulated vascular plant root growth in shallow soils. Greater root biomass, root length, root surface area, and numbers of root branches in moss-covered than in moss-free soils suggested that mosses at the soil surface can have a significant influence on vascular plant activity belowground, particularly for forb species. These results are important in extending our understanding of aboveground influences on belowground functioning in these systems (Chapter 1); in clarifying rooting patterns in a system where roots contribute significantly to peat formation; and in suggesting additional mechanisms by which rich fen mosses may influence vascular plant species success in these diverse wetlands (Chapter 3).

While I am unaware of other studies investigating the influence of mosses on root morphology or distribution, I would expect moss effects on root activity to occur primarily through mediation of two environmental factors that strongly influence root growth: soil saturation and nutrient availability. Mosses also may influence plant rooting through factors such as pH or temperature (Gregory 2006), but this seems less likely here. Prior work showed that rich fen mosses did not affect pH in surface soils (Chapter 1), and moss insulation of the soil surface likely would reduce rather than stimulating additional root growth (Gregory 2006, Chapter 1). Greater redox potential underlying mosses (Chapter 1), however, indicated more oxidized soil conditions that might stimulate the increased root growth observed in this study. Additionally, greater forb tissue phosphorus concentrations where mosses occurred suggested greater availability of phosphorus underlying mosses (Chapter 1), which could drive root proliferation as a foraging response. While I cannot isolate redox from nutrient effects

through the moss removal experiment and field survey described here, I use observed patterns of root morphological and topological response to mosses to explore the relative importance of these mechanisms.

Root proliferation in response to more oxidized soils. In previous work, more oxidized soil conditions underlying mosses appeared to be a key driver both of increased phosphorus supply to plants (through greater microbial phosphorus mineralization) and greater phosphorus acquisition (through increased plant vigor leading to greater root phosphatase activity and AMF colonization of forb roots) (Chapter 1). I therefore hypothesized that more oxidized soil conditions underlying mosses also might promote root growth in shallow soils. Supporting this hypothesis, root and shoot biomass for *Solidago patula* seedlings were significantly greater where mosses occurred than where they had been removed by the end of the second growing season (Figure 2-4), with no concurrent change in the ratio of root to shoot biomass (Figure 2-5), implying increased vigor of seedlings planted in moss-covered soils. In the field survey, root biomass for existing vegetation also was greater in shallow soils immediately underlying mosses (Figure 2-1). In wetland habitats, lower root biomass is common where water levels are high (e.g., Kotowski et al. 2001, Kercher and Zedler 2004, Luo and Xie 2009). Kotowski et al. (2001) and Kercher and Zedler (2004) found this to be true for fen plant species, specifically, and Kercher and Zedler (2004) determined that the most flood-sensitive of the 16 wetland species they tested were those diagnostic of groundwater-fed, nutrient-poor fens. While water levels in the moss removal experiment typically remained at or below the soil surface, soil oxygen demand can affect root functioning even without submergence, which may have a greater effect on photosynthetic activity and growth (van Bodegom et al. 2008). Additionally, levels of reduced iron were significantly higher in moss removal than in moss control plots (Chapter 1), suggesting the possibility of iron toxicity under

reducing conditions (Snowden and Wheeler 1993). Therefore, although moss effects on redox potential were limited to shallow soils, they appeared sufficient to generate a root biomass response.

Measures of root morphology where mosses occurred also were consistent with a response to more oxidized soils. Both the existing vegetation in the field survey and the test seedlings of forb species from moss control and moss removal plots showed greater root length underlying mosses (Figure 2-1, 2-6). In addition to greater root length overall, external root links were longer, average root diameter was marginally lower, more root branches were present, and specific root length was greater for test seedlings of forb species from moss-covered soils (Figure 2-6, 2-7, 2-8, and 2-9). Water-logging has been shown to decrease lateral root development (Visser et al. 1996, Bouma et al. 2001b), which would be expected to reduce external link length and root length overall. Where water levels were high in a salt marsh system, Bouma et al. (2001b) found first-order lateral roots to be shorter and the main root axis to make a larger contribution to total root length. A larger root diameter may promote flood resistance (Visser et al. 1996) and tends to lower specific root length in reducing soils. Although moss-generated differences in redox potential typically did not include seedling submergence, root morphological differences between treatments were consistent with the expected effects of differences in water level.

Root proliferation in response to localized phosphorus availability. Previous work suggested that more oxidized soils underlying mosses may promote greater soil biological activity and therefore drive phosphorus dynamics, including phosphorus acquisition by plants (Chapter 1). Greater root proliferation due to improved plant vigor could be an additional contributor to greater phosphorus acquisition from moss-covered soils; greater plant phosphorus uptake implies greater localized phosphorus availability underlying mosses that might stimulate a root foraging response. Either an

excess or a deficit of a limiting nutrient may stimulate root proliferation, depending on the scale considered and on root structural characteristics (Hutchings and John 2003, Hodge 2004, Ryser 2006). Where nutrient availability overall is low, root biomass or length may be correspondingly high to enable sufficient nutrient uptake (Hutchings and John 2003, Ryser 2006). Where soil nutrient availability is heterogeneous, as is usually the case, roots also may proliferate into localized patches of greater nutrient availability in a foraging response (Hodge 2004, 2006 and references therein). Such responses particularly may benefit the plant when they involve less mobile nutrients such as phosphorus, rather than nutrients such as nitrogen that may leave the system before changes in root morphology can influence uptake (Robinson 1996, Fitter et al. 2002, Hutchings and John 2003, Hodge 2004). Plant root foraging responses have been well demonstrated generally, and multiple studies have shown root morphology, architecture, or distribution to respond to phosphorus availability, specifically (Liao et al. 2001, Fitter et al. 2002, Yano and Kume 2005, Hill et al. 2006).

Because forb seedling phosphorus uptake was greater from moss-covered soils than where mosses had been removed (Chapter 1), one of two phosphorus-driven root responses seemed possible: (1) root proliferation could be lower underlying mosses because fewer roots were needed to enable sufficient phosphorus uptake, or (2) root proliferation could be greater immediately underlying mosses in a foraging response to greater localized phosphorus availability. Because seedling root biomass, measures of length, and measures of branching all were greater or unchanged in moss control relative to moss removal plots, the first possibility appeared not to operate in this case.

Thus, as well as responding to more oxidized soil conditions underlying mosses, roots could be proliferating to take advantage of localized areas of greater phosphorus availability within the zone of moss influence. In the field survey of existing vegetation, greater root biomass and length immediately underlying mosses

(Figure 2-1) were consistent with work on rooting patterns in common bean, which showed that bean roots were distributed more shallowly (where nutrients tended to be more abundant) in soils that were phosphorus-limited overall (Ge et al. 2000, Liao et al. 2001, Rubio et al. 2003, Liao et al. 2004). Similarly, greater root length, numbers of root branches, and specific root length for *Solidago patula* and *Lycopus uniflorus* test seedlings in moss control plots (Figure 2-6, 2-7, and 2-9) were consistent with studies in which longer, thinner roots were considered advantageous in exploring nutrient patches (Ryser 2006 and references therein, although noting that this relationship does not always hold). This same set of measures for *Carex hystericina* seedlings showed no difference in response to mosses, but this was expected given the deeper root systems and lower morphological plasticity observed both for graminoids generally (Taub and Goldberg 1996, Bouma et al. 2001a, Bouma et al. 2001b) and for another sedge species typical of low-nutrient fens (Perez-Corona and Verhoeven 1996, 1999). Results for *Carex hystericina* in comparison to the two forb species are discussed in more detail below.

While these measures of root response were consistent with the hypothesis that roots proliferated to exploit areas of localized phosphorus availability underlying mosses, other measures were not. As discussed above, root biomass for *Solidago patula* seedlings in moss control plots was greater by the end of the second growing season (Figure 2-4), but the lack of a corresponding change in the ratio of root to shoot biomass (Figure 2-5) suggested that seedlings were simply growing more overall where mosses occurred (and thus demonstrating greater vigor), rather than concentrating biomass in localized areas belowground. Similarly, root branching patterns as indicated by the topological index (TI) showed no difference between moss-covered and moss-free areas (Figure 2-10). Lower values of TI indicate a dichotomous root branching pattern that may be advantageous for nutrient foraging

because of increased potential for soil exploration and possibly lower carbon construction cost (Fitter 1987, Fitter and Stickland 1991, Fitter et al. 1991, but see discussion of carbon costs in Bouma et al. 2001b). Increased dichotomous branching has been observed under greater plant competition or patchy resource availability (Fitter and Stickland 1992, Taub and Goldberg 1996, Bouma et al. 2001b). In contrast, a herringbone pattern (with reduced lateral branching) is thought to be more typical of lower nutrient conditions (although this may be particularly evident for mobile nutrients such as nitrogen). Thus, if seedling roots proliferated to take advantage of greater localized phosphorus availability underlying mosses, seedlings in moss control plots would be expected to have a lower TI value. Given that this was not evident, observed differences in root growth may not have been driven primarily by phosphorus foraging, even in this phosphorus-limited system.

More dichotomous root branching patterns potentially could be indicative of more oxidized soil conditions as well as greater localized nutrient availability; for example, Bouma et al. (2001b) suggested that plants from wetter areas of a salt marsh may have demonstrated herringbone rather than dichotomous root branching in part to maximize nutrient uptake where availability was low, and in part to reduce oxygen loss and minimize damage by phytotoxins under reducing conditions. The lack of a significant difference in TI between treatments in this study therefore may have alternative explanations. Fen plants may be less plastic than other species in their topology, although this seems unlikely given observed morphological variation and Jansen's work demonstrating highly selective root placement for wetland plant species (Jansen et al. 2005, Jansen 2007). Root branching patterns have been less frequently examined for wetland species, and the increased herringbone branching observed by Bouma et al. (2001b) may be restricted to species of flooded environments. It is also possible that semi-random root selection for topological analysis in this study included

roots from depths beyond the zone of moss effect. Further clarification of root branching responses to interactions between soil saturation and phosphorus availability in these systems would require further experimental work under controlled conditions.

Differential responses by sedge vs. forb species

Root morphological responses to moss removal clearly differed between seedlings of the two forb species examined (*Solidago patula* and *Lycopus uniflorus*) and the sedge species (*Carex hystericina*). In moss-covered vs. bare soils, the two forb species demonstrated significantly greater root length, greater specific root length and specific root area, a marginally lower average root diameter, and greater numbers of root tips (Figure 2-6, 2-7, 2-8, and 2-9). In contrast, *Carex hystericina* showed no treatment effects for any of these measures. These results are consistent with studies in both wetland (Bouma et al. 2001a, Bouma et al. 2001b) and upland systems (Fitter and Stickland 1992, Taub and Goldberg 1996), where forb species showed a level of morphological plasticity not present in grasses (but for an exception see Smilauerova and Smilauer 2006). As expected, the roots of *Carex hystericina* responded similarly to those of many other graminoids. More specifically, the lack of a moss removal effect on root biomass or specific root length for *Carex hystericina* was consistent with the lack of a phosphorus effect on the same measures for *Carex lasiocarpa*, another species typical of low-phosphorus fens, although *Carex acutiformis* (a species typical of higher nutrient conditions) showed some plasticity in these measures (Perez-Corona and Verhoeven 1996, 1999). The lack of root morphological response for *Carex hystericina* to differences in redox potential underlying mosses also would be expected, as sedges tend to show greater adaptation to saturated soils than do forb species.

The differential responses to the presence or absence of mosses between *Carex hystericina* and the two forb species may help explain patterns of root morphology for naturally occurring, sedge-dominated vegetation in the field survey. In the field survey at depths of 0-1 cm, specific root length and specific root area were lower and average root diameter higher in areas where mosses occurred than where they were naturally absent (Figure 2-2 and 2-3). This was opposite the pattern observed for the two forb species but matched the direction of the trend observed for *Carex hystericina*, which while not significant on a seedling scale, may be important when integrated across a sedge-dominated fen.

In contrast to measures of specific root length, specific root area, and diameter, field survey measures of root biomass and length in shallow soils for the existing, sedge-dominated vegetation matched patterns observed for the two forb species rather than those for *Carex hystericina*: root biomass and length were greater at a depth of 0-1 cm underlying mosses than where mosses naturally were absent (Figure 2-1). These results suggest that the effects of mosses on these very shallow soils may be of sufficient magnitude to stimulate root proliferation even for the less morphologically plastic sedge species that comprise the majority of the root biomass. At greater depths, where moss influences would be reduced, differences between sedge-dominated roots from moss-covered and moss free areas equalized to match the results found for *Carex hystericina* seedlings.

Mosses promoted forb phosphorus uptake

The presence of mosses significantly affected tissue phosphorus concentrations for seedlings of the two forb species: for *Solidago patula* and *Lycopus uniflorus*, shoot and root tissue phosphorus concentrations were greater or marginally greater where mosses occurred than where they had been removed, excepting only root tissues

for *Solidago patula* at LF (Figure 2-12). Prior work suggested that greater phosphorus acquisition by forbs was driven by more oxidized soils underlying mosses, with subsequently greater microbial activity (resulting in greater phosphorus release) and increased plant vigor (promoting root phosphatase activity and root colonization by AMF) (Chapter 1). In a greenhouse experiment, the presence of AMF in combination with drier soils increased both plant tissue phosphorus concentrations and root and shoot biomass for *Solidago patula* and *Lycopus uniflorus* (Weishampel 2005). In more oxidized soils underlying mosses, increased plant vigor and associated root growth may promote greater phosphorus acquisition through several interacting mechanisms—including direct phosphorus uptake via proliferating roots—that together result in greater phosphorus uptake by forb species.

Similar to root morphological measurements, differences in plant tissue phosphorus concentrations were not evident for *Carex hystericina* roots or shoots (Figure 2-12). They also were not reflected in root tissue phosphorus concentrations for existing vegetation from moss-covered vs. naturally moss free areas (Figure 2-11), which is reasonable given that sedge roots (of *Carex hystericina* and other species) would be expected to comprise most of the root biomass for the existing vegetation.

Summary and conclusions

Rich fen mosses clearly influenced vascular plant activity belowground by stimulating greater root proliferation relative to areas where mosses did not occur. This was particularly evident in surface soils for existing vegetation and for seedlings of the two forb species examined. Because many root morphological measures (length, specific root length, diameter, indicators of branching) were consistent with proliferation in response either to more oxidized soils or to localized increases in phosphorus availability underlying mosses, such measures could not distinguish

cleanly between these two mechanisms. Measures of greater second-year root and shoot biomass for *Solidago patula* seedlings where mosses occurred, however, suggest that more oxidized soils promoted greater plant vigor overall. Additionally, consistent TI values between moss control and moss removal plots suggested comparable root branching patterns rather than a localized foraging response underlying mosses. Root proliferation where mosses occur may be a combined response to more oxidized soils and localized increases in phosphorus availability immediately underlying mosses (Chapter 1), with soil redox conditions again as a predominant driver. Thus, greater forb seedling tissue phosphorus concentrations where mosses occur could derive from more vigorous plants growing in more oxidized soils, able to obtain phosphorus in a low-phosphorus environment through higher root phosphatase activity, greater root colonization by AMF, and root proliferation in response to localized, microbially driven increases in phosphorus availability (Chapter 1). Further clarifying the relative influence of these environmental factors on plant rooting patterns would require additional experimental work.

Overall, this work suggests that rich fen mosses covering the peat surface have an important influence on root activity belowground, which has broader implications for peat formation as well as for individual plant species success. This extends our understanding of aboveground influences on belowground functioning in rich fens and provides a new example of the key functional role of a frequently overlooked plant group. The differential effects of mosses on root growth and morphology for seedlings of forb and sedge species have important implications for understanding controls on plant species composition in these species-rich wetlands. In rich fens where shallowly rooted forbs comprise much of the high species diversity, moss effects on root growth may play a significant role in helping these species to succeed in a saturated and phosphorus-limited environment.

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CHAPTER 3

MOSSES FACILITATE FORB SPECIES ESTABLISHMENT AND DIVERSITY IN FOUR RICH FENS IN NEW YORK AND MICHIGAN

ABSTRACT

Positive interactions between species increasingly are recognized for their importance in plant communities; however, questions remain regarding the relationship between facilitation and environmental stress gradients, and the contributions of positive species interactions to the species composition and diversity of a system. In rich fens in New York and Michigan, which are characterized by high forb species density and conditions of nutrient limitation and soil saturation, I hypothesized that mosses covering the peat surface would facilitate seedling establishment and increase species density of vascular plants. I used a field survey and a moss removal experiment to investigate how rich fen mosses influenced forb seedling emergence, survival, and establishment, and how these influences were reflected in plant species composition and species density. The presence of mosses resulted in greater seedling survival for two diagnostic rich fen species (*Solidago patula*, *Packera aurea*) and two wetland generalists (*Eupatorium maculatum*, *Mentha x piperita*). Both in the field survey and under experimental conditions, abundance of naturally established forbs was greater where mosses occurred, and this difference persisted when individuals grew into larger (> 5 cm) size classes. Total forb cover and forb species density also were greater in the presence of mosses, while graminoids showed no response. This work provides additional evidence for the importance of positive plant species interactions in environmentally stressful habitats, and suggests the possibility (following recent theoretical work) that fen mosses promote forb diversity by enabling wetland generalists to become established under otherwise

stressful reducing or low-phosphorus conditions. Conservation and restoration of high forb species density in rich fens may require particular attention to retention or establishment of the moss layer.

INTRODUCTION

The mechanisms underlying variation in plant species diversity are a question of long-standing ecological interest. In one of the earliest integrations of factors influencing the number of species in a given area, Grime (1973a, 1973b, 1979) suggested that diversity would be greatest at intermediate levels of community biomass, where environmental conditions were sufficient to support growth, but where neither biomass, environmental stress, nor disturbance were so high as to exclude either less competitive or less tolerant plant species. While many hypotheses regarding the mechanisms maintaining species diversity have been proposed (reviewed in Grace 1999), Grime's hump-backed model continues to be influential; however, a growing body of work suggests that it may not be complete in its consideration of plant species interactions. In addition to the competitive biotic interactions emphasized in Grime's model, a growing consensus holds that positive interactions between plant species also should be incorporated into ecological theory (Bruno et al. 2003, Brooker et al. 2008).

Employing a perspective similar to that of the hump-backed model, efforts to understand the principles governing facilitation have focused particularly on the relationship between positive species interactions and environmental or disturbance gradients. The stress-gradient hypothesis proposes that facilitation may be greatest in areas of high environmental stress such as alpine zones or deserts, and many studies support this idea (reviewed in Bertness and Callaway 1994, Brooker et al. 2008), although some controversy remains to be resolved (Maestre et al. 2005, Brooker et al.

2008). Recent approaches toward understanding mechanisms influencing species density have proposed an alignment of thinking about diversity and facilitation: while competitive interactions may result in lower species densities at high community biomass and low stress or disturbance, facilitative interactions may help maintain higher species densities at lower biomass and greater environmental stress (Hacker and Gaines 1997, Michalet et al. 2006). In particular, Michalet et al. (2006) suggest that positive interactions may expand the range of competitive, stress-intolerant species into regions of moderate to high environmental stress, thereby increasing diversity in this zone. Under this model, biotic interactions would influence both ends of Grime's hump-backed curve.

I investigated these concepts in rich fens, which are ideal systems for exploring ways in which facilitative interactions may influence species density. Rich fens are peatlands fed primarily by groundwater high in minerals such as calcium or magnesium, and they harbor disproportionately high levels of plant species diversity and rarity given their small geographic extent (Bedford and Godwin 2003). Community biomass in rich fens is typically low to intermediate, and plants may experience stress by nutrient limitation, soil saturation and reducing conditions in most of the root zone, or potentially toxicity from reduced sulfur or iron (Snowden and Wheeler 1993, 1995). Thus, rich fens are both of particular interest for exploring mechanisms that maintain high species density, and they occupy the region of the hump-backed curve where environmental stress may be high and facilitation therefore likely to occur.

Several interacting explanations have been proposed to help clarify patterns of plant species diversity in rich fens, including phosphorus limitation or nitrogen co-limitation suppressing dominant species (Boyer and Wheeler 1989, Bedford et al. 1999, Bedford and Godwin 2003); mycorrhizal support of subordinate fen species

within a non-mycorrhizal sedge matrix (Weishampel and Bedford 2006); and heterogeneity in phosphorus availability generated by groundwater-driven redox processes, associated with plant species composition at a site scale (Bailey 2006, Boomer and Bedford 2008a, b). In an analysis of data from 16 fens in New York, Gusewell et al. (2005) determined that the relationship between species density and above-ground biomass was generally consistent with a hump-backed curve, but variability in the relationship was high, and biomass, pH, and the ratio of nitrogen to phosphorus in the vegetation explained only 50% of the variation in species density among the sites. It seems possible that facilitation could influence plant species composition and diversity in these systems under conditions of small-scale environmental heterogeneity, but such mechanisms rarely have been examined.

Consideration of the vegetation structure of rich fens particularly suggests that positive plant species interactions may be important in these wetlands. Many rich fen vascular plant species are small, shallowly rooted forbs that would be unlikely to persist under conditions of strong competition. Rich fens also include a number of less stress-tolerant, generalist forb species typical of more competitive wetland systems (Bedford and Godwin 2003); Michalet et al. (2006) hypothesized these to be the species helped most by positive interactions with stress-tolerant species. Fen forb species occur within a matrix of small-stature but dominant sedge species, generally with deeper root systems. Fen forb species typically are rooted immediately below a layer of mosses that cover the peat surface, and this association between forb species and mosses suggests the possibility that mosses might facilitate forb survival and growth in rich fens.

Mosses have been shown to have both positive and negative effects on seed germination, seedling emergence, and seedling survival (During and van Tooren 1990). Positive or mixed effects of mosses on vascular plants have been reported from

systems such as high-arctic meadows (Sohlberg and Bliss 1987), serpentine seeps (Freestone 2006), limestone grasslands (Ryser 1993), and a fen meadow supporting a rare species (Overbeck et al. 2003). In several studies of herbaceous wetlands and grasslands, however, the presence of mosses primarily has inhibited seedling emergence and survival (Kotorova and Leps 1999, Zamfir 2000, Otsus and Zobel 2004, Spackova and Leps 2004, Stammel et al. 2006). This inhibition typically has been attributed to competitive interactions between mosses and vascular plants, such as blocking of seeds from reaching the soil surface, direct inhibition by a thick moss mat, or reduction of light intensity. Positive effects of mosses in some cases have been attributed to prevention of seed loss (i.e., seed retention, Freestone 2006), seed predation, or herbivory (During and van Tooren 1990). In a majority of studies in extreme environments, however, positive moss effects have been attributed to alleviation of environmental constraints, suggesting that positive effects of mosses often may outweigh competitive effects under conditions of environmental stress.

In rich fens, mosses have the potential to alleviate environmental stress in related ways that may improve seedling emergence and survival and therefore influence plant species composition and diversity overall. Mosses can influence physicochemical conditions in their immediate environment, including temperature, moisture, pH, and nutrient availability (Vitt 2000, Sedia and Ehrenfeld 2003, Turetsky 2003, Cornelissen et al. 2007, Gornall et al. 2007). Previous work showed rich fen mosses to increase redox potential in surface soils (Chapter 1), which would be expected to improve conditions for seedling emergence and establishment (e.g., Fraser and Karnezis 2005). Greater redox potential also was associated with lower concentrations of reduced iron underlying mosses (Chapter 1), reducing the risk of iron toxicity to seedlings (Snowden and Wheeler 1993, 1995). Additionally, phosphorus uptake by fen forb species was greater where mosses occurred, suggesting

that forb seedlings rooted in mosses might be more able to tolerate the phosphorus-limited conditions typical of fens (Chapter 1).

Because mosses affected physicochemical conditions in rich fen soils in directions likely to alleviate stress to vascular plant seedlings, I hypothesized that rich fen mosses would improve seedling emergence and survival. Given that mosses frequently cover the soil surface in highly diverse rich fens, I expected effects on seedling establishment to be reflected in vascular plant species composition and diversity overall. The forb component of the fen vegetation could be more subject to these influences than would the dominant, clonally spreading sedges because forb species are more likely to establish from seed, and because sedge species tend to be more tolerant of reducing conditions. Additionally, based on the Michalet et al.(2006) diversity-facilitation model, I expected wetland generalist species as well as diagnostic fen species to be facilitated by mosses. I used field surveys and a moss removal experiment to explore these hypotheses, asking the following specific questions: (1) Do rich fen mosses facilitate forb seedling emergence and survival? (2) Do fen specialist and wetland generalist species respond similarly to the presence of mosses? and (3) Are moss effects on forb seedlings reflected in vascular plant species composition and diversity overall?

METHODS

Study sites

I performed field surveys of seedling survival and abundance and assessed responses of the vascular plant community to experimental moss removal at subsets of five rich fens in central New York and Michigan. Larry's Fen (LF), Clark Street Extension Fen (CSE), Fish Fen (FF), and Belle School Fen (BSF) are in the vicinity of Cortland, NY (42.6°N, 76.2°W). Mott Road Fen South (MRFS) is located on the

property of the Fort Custer Training Center near Augusta, MI (42.3°N, 85.3°W). LF and MRFS are sloping fens fed by hillside seepage, with vegetation dominated by a matrix of *Carex* L. (sedge) species, *Dasiphora floribunda* (Pursh) Raf. (shrubby cinquefoil), mixed forbs, and the moss species *Campylium stellatum* (Hedw.) C. Jens. CSE has a similar plant species composition but slopes more gently and is dominated by *Campylium stellatum* and *Sphagnum warnstorffii* Russ. in the moss layer. BSF is a sloping fen dominated by *Typha latifolia* L. (broadleaf cattail), *Solidago patula* Muhl. ex Willd. (roundleaf goldenrod), *Packera aurea* (L.) A. & D. Löve (golden ragwort), additional mixed forbs, and diverse moss species including *Campylium stellatum*, *Brachythecium* sp. Schimp., *Sphagnum warnstorffii*, and others. FF is a sloping depressional fen dominated by *Typha angustifolia* L. (narrowleaf cattail), diverse forbs, and the moss species *Calliergonella cuspidata* (Hedw.) Loeske and *Campylium stellatum* plus a number of less frequent mosses. (Nomenclature follows USDA and NRCS (2009).) The moss layers in the four sites (which include a minimal abundance of liverwort species) ranged in depth from approximately 3-5 cm and ranged in coverage from nearly continuous (85-95% cover) to highly patchy (20-90% cover).

LF, CSE, and BSF occur within predominantly agricultural watersheds, and CSE has a known history of past cattle grazing. While FF is surrounded mainly by steeply sloping forest and is located within a natural area, it served as a millpond during the late 1800s and has accumulated a minimum of 25-35 cm of new peat since abandonment. MRFS is surrounded by young forest, and the site underwent prescribed burns in the springs of 2004 through 2006. At all five sites, water levels remain within 5-10 cm of the peat surface throughout the growing season.

Field surveys

To compare the abundance and diversity of forb seedlings in the presence and absence of mosses, I established five blocks of three paired 10x10-cm plots in moss-covered and adjacent moss-free areas at LF and BSF in September 2005 and at CSE and MRFS in September 2006. The blocks at each site were established with paired plots to control for variability in environmental conditions across the sites. Within each plot, I recorded the number of forb seedlings by species and height class (<2 cm, 2-5 cm, 5-10 cm, and >10 cm).

Within the paired areas where mosses occurred or naturally were absent at LF and BSF, I tagged naturally occurring germinants and tracked their survival and growth through the summer of 2006. In both moss-covered and moss-free areas, I marked individuals for each of two diagnostic fen species in New York, *Solidago patula* and *Packera aurea* (Reschke 1990), and a wetland generalist hybrid of two non-native species, *Mentha x piperita* (pro sp.) [*aquatica* × *spicata*] (peppermint). I indicated seedling locations with a plastic marker adjacent to each seedling. Every three weeks from May 25 through October 25, 2006, I recorded seedling presence and height for each tracked individual. Where possible, I also recorded potential causes of seedling mortality such as herbivory or inundation.

Moss removal experiment

In September 2004 at MRFS, in June 2005 at LF and FF, and in June 2006 at CSE, I established five sets of paired 0.5x0.5-m randomly assigned moss removal and moss control plots at each fen. Treatments within each pair included a moss control plot with no manipulation and a moss removal plot in which mosses were removed while all other plants (predominantly forbs and graminoids) were left in place. In each treatment block at LF and FF, I also established a disturbance control plot in which

mosses were removed and replaced (in place) to ensure that the effects of moss removal were not caused by soil disturbance. In removal and disturbance control plots, all living mosses were removed or removed and replaced by hand, using a definition of living moss as green tissue (ranging from 2-3 cm depth) plus firmly attached brown tissue (1-2 cm). Treatment blocks were established in regions of each fen where the dominant moss species were "brown" mosses (typically in the family *Amblystegiaceae*), predominantly *Campylium stellatum* or *Calliergonella cuspidata*, with a minimum total moss cover of 80%. The moss removal treatment was maintained twice per growing season to counteract moss re-colonization.

In July 2006-2007 at LF, FF, and MRFS and in July 2007 at CSE, I assessed the response of vascular vegetation to the presence of mosses by counting forb seedlings and estimating percent cover for all plant species in moss control, moss removal, and disturbance control plots. To determine seedling abundance, I recorded the number of forb seedlings by species and height class (<2 cm, 2-5 cm, 5-10 cm, and >10 cm) in each of three randomly placed 10x10-cm subplots per moss control or removal plot. Percent cover estimates for graminoid and forb species were made visually for each 0.5x0.5-m plot.

To investigate the effects of mosses on seedling emergence and survival, in November 2006 I randomly placed a 10x10-cm seed germination subplot in each moss control and moss removal plot at LF. Within each subplot, I distributed evenly 50 seeds of *Solidago patula* (a typical rich fen species) and 50 seeds of *Eupatorium maculatum* L. (spotted joeypyeweed; a wetland generalist species) collected at LF in October 2006. Beginning on April 30, 2007, I marked each emerging seedling with a plastic, colored toothpick on a 2x2-cm grid system. I recorded presence, species, and height for each seedling every five days from April 30 through May 30 and then monthly through October 2007.

Data analysis

I analyzed data from the moss removal experiment and the field survey of forb seedling abundance using generalized linear mixed models (proc MIXED, SAS version 9.1). For the field survey, I analyzed the effects on seedling count and species density of block (random factor), site, and the presence or absence of moss (fixed factors). For the moss removal experiment, I separated the analysis into two stages, first analyzing the effects on seedling count, percent cover, and species density of experimental block (random factor), site, and treatment (fixed factors) by year for three sites (FF, LF, MRFS) in 2006 and four sites (FF, LF, MRFS, CSE) in 2007. I then used a repeated measures analysis to analyze a similar model for FF, LF, and MRFS alone, with year as the repeated measure for each site-block-treatment combination. To analyze seedling emergence and survival data, I examined the effects on percent emergence and percent survival of block (random factor), site, treatment, and species (fixed factors). For both the field survey and the moss removal experiment, I used a log or $\log(x+1)$ transformation where needed to satisfy model assumptions of normality and homogeneity of variance, and I used Satterthwaite's approximation to estimate denominator degrees of freedom (Littell et al. 2006).

For the field survey of seedling survival in moss-covered and naturally moss-free areas, I used binary logistic regression to assess whether survival for each species was more likely where mosses occurred. Differences in height for surviving seedlings of each species were analyzed in proc MIXED using a 1-way model with the presence of moss as a fixed factor. Because of the uneven distribution of seedlings across blocks and sites, I did not include block or site factors in this analysis.

RESULTS

Seedling emergence, survival, and establishment

In the moss removal experiment, mosses significantly influenced both seedling emergence and seedling survival. For seedling emergence, however, the presence of mosses affected the two forb species considered in opposite ways. For *Eupatorium maculatum*, mosses inhibited seedling emergence, and percent emergence in moss removal plots was approximately twice that of moss control plots (Figure 3-1a). In contrast, for *Solidago patula* the presence of mosses improved seedling emergence, and percent emergence in moss control plots was nearly three times the level of removal plots (Figure 3-1a). In spite of this difference in emergence of seedlings, seedling survival at the end of the 2007 growing season for both species was 2-3 times greater where mosses occurred than where they had been removed (Figure 3-1b). Percent survival of *Solidago patula* seedlings in moss control plots was approximately twice that of *Eupatorium maculatum* seedlings, reflecting the higher initial emergence of *Solidago patula* seedlings in moss-covered soils (Figure 3-1b).

Patterns observed under experimental conditions also were evident in the field survey. For naturally occurring seedlings of *Solidago patula*, *Packera aurea*, and *Mentha x piperita*, the likelihood of seedling survival by September 2006 was significantly greater where mosses occurred than where they were naturally absent (Figure 3-2a). The species with the lowest seedling survival, *Mentha x piperita*, is abundant but not native to the fens. Growth of the tracked seedlings reflected the differences in survival for only one of the three species: the average height of *Solidago patula* seedlings was consistently greater where mosses occurred than where they were absent (Figure 3-2b).

Corresponding to the patterns of seedling emergence and survival for individual species, the total number of naturally established forb seedlings and larger

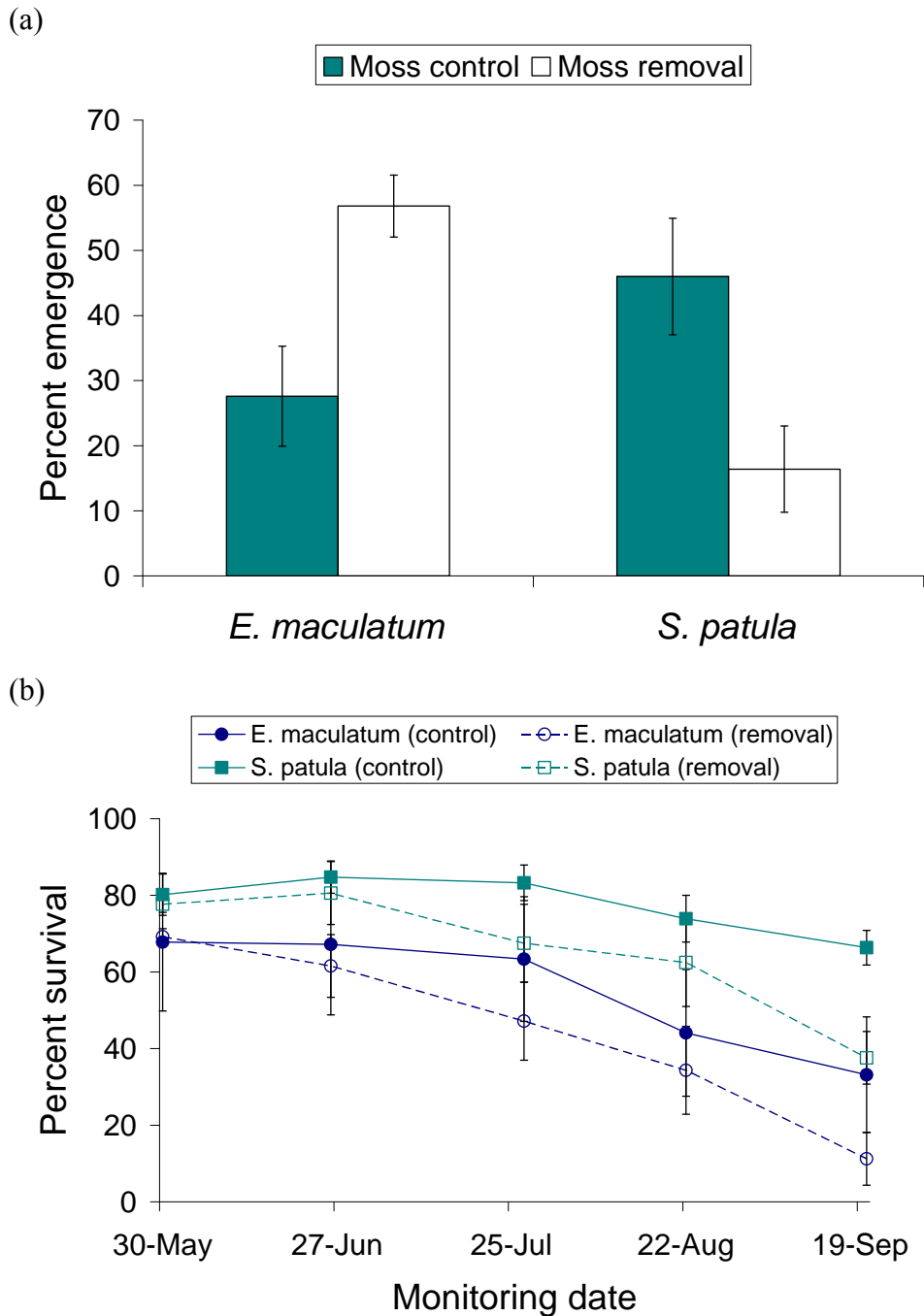


Figure 3-1. Effects of moss removal on (a) percent seedling emergence ($p \leq 0.01$ for each species) and (b) percent survival of emerged seedlings through September 2007 ($p < 0.05$ by September; the trend continued into October but was complicated by seedling senescence). Seeds of *Solidago patula* and *Eupatorium maculatum* were collected in October and planted in moss control (solid bars and symbols) and moss removal plots (open bars and symbols) at Larry's Fen (LF) in November 2006. Error bars \pm SE.

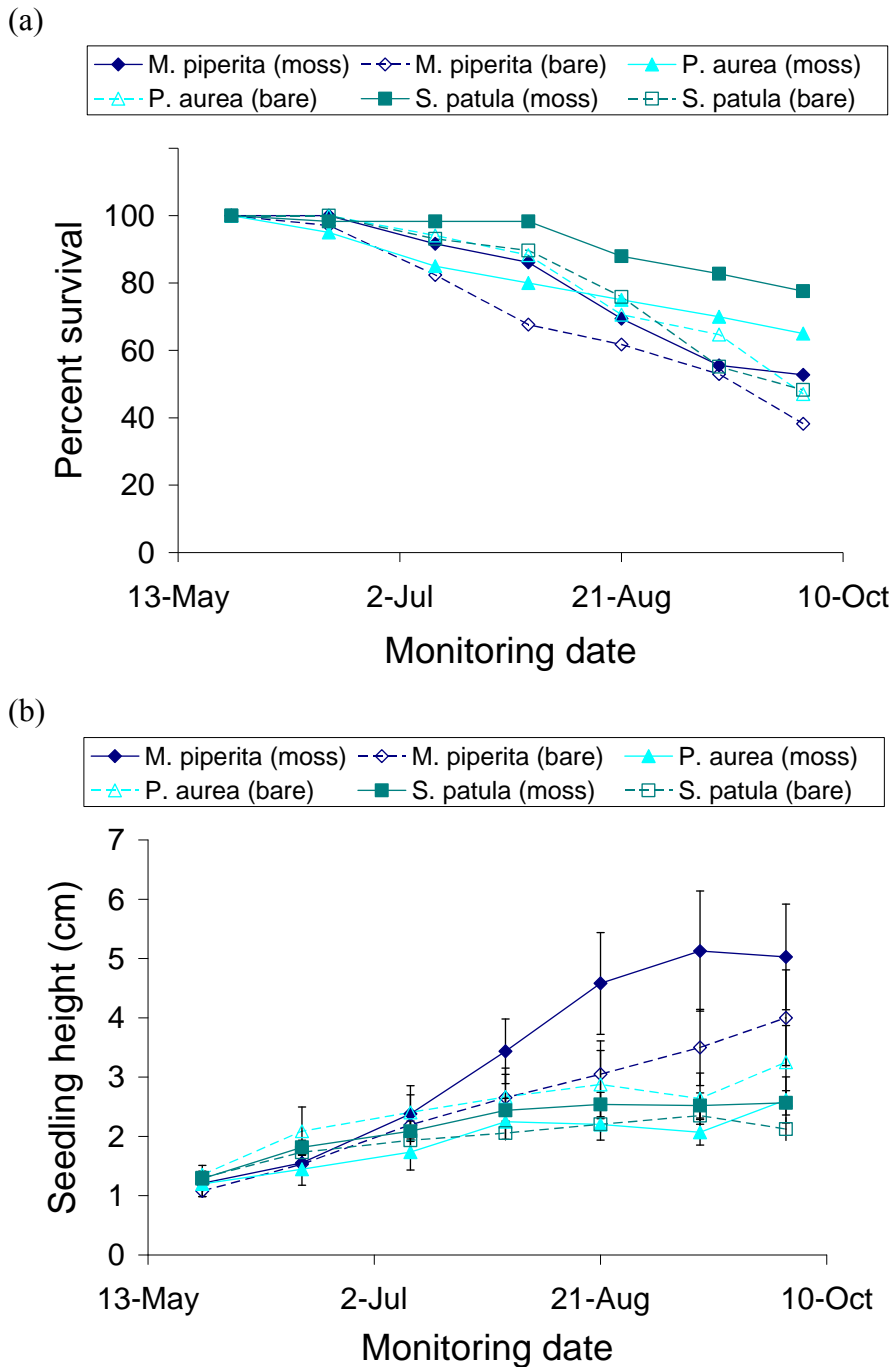


Figure 3-2. Effects of the presence or natural absence of mosses on (a) percent seedling survival and (b) seedling height through summer 2006 ($p < 0.05$ for September for *Solidago patula* only) for naturally established seedlings of three forb species at Belle School Fen (BSF) and Larry's Fen (LF). Analysis of survival was based on a binary logistic regression indicating that by September, the chance of survival was greater where mosses occurred ($p < 0.001$). Filled symbols and solid lines indicate that mosses were present; open symbols and dashed lines indicate that mosses were absent.

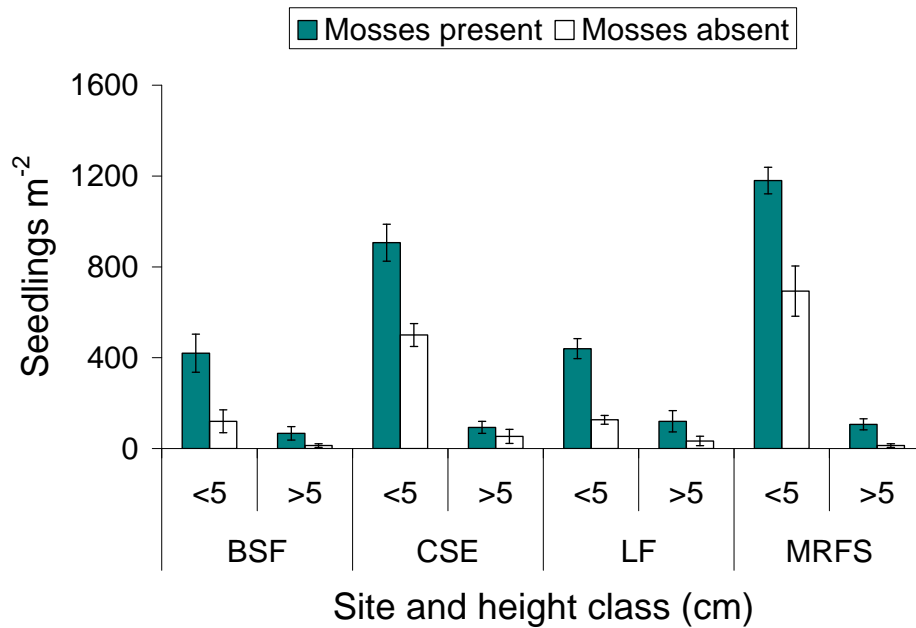
plants also was greater where mosses occurred than where they were absent, both in the field survey and in the moss removal experiment (Figure 3-3). The total number of individuals was greater in moss-covered than in moss-free areas for both newly established seedlings (height class <5 cm) and for larger plants (height class >5cm), although the magnitude of the difference and the total number of individuals were lower for the larger size class (Figure 3-3). The total number of individuals did not differ between moss control and disturbance control plots at FF and LF, indicating that the effects of moss removal were not a result of soil disturbance (n.s.).

Plant species composition and diversity

The effects of mosses on seedling emergence, survival, and establishment were reflected in the plant species composition overall. While responses varied somewhat by species and sampling year, individuals of all species were either more abundant or equally abundant in moss control plots than in moss removal plots (Figure 3-4). (A similar analysis was not performed for the field survey because insufficient newly emerged seedlings were identified to species.) Corresponding to rates of seedling emergence and seedling survival, *Solidago patula* individuals were more abundant overall than those of *Eupatorium maculatum*, and both species demonstrated greater numbers of naturally established plants where mosses occurred (Figure 3-4). The greater abundance of forb individuals associated with mosses also was evident in percent cover estimates; total percent cover for forb species was significantly greater where mosses occurred than where they had been removed (Figure 3-5). In contrast, total graminoid cover (consisting primarily of sedges) did not show a treatment effect (Figure 3-5).

Species density estimates reflected the greater establishment of forbs in moss-covered than in moss-free areas. Forb species density calculated from individual plant

(a) Field survey



(b) Moss removal experiment

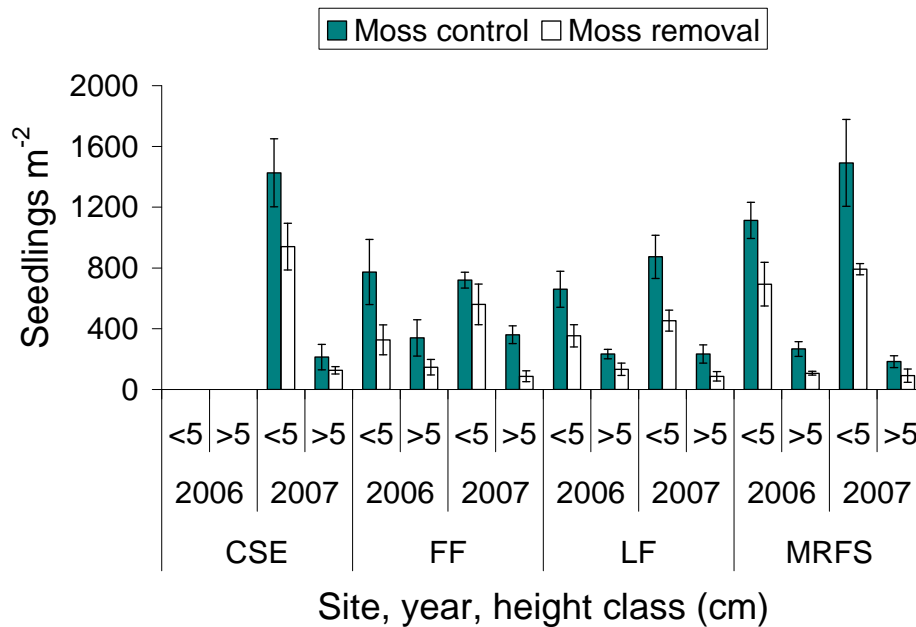
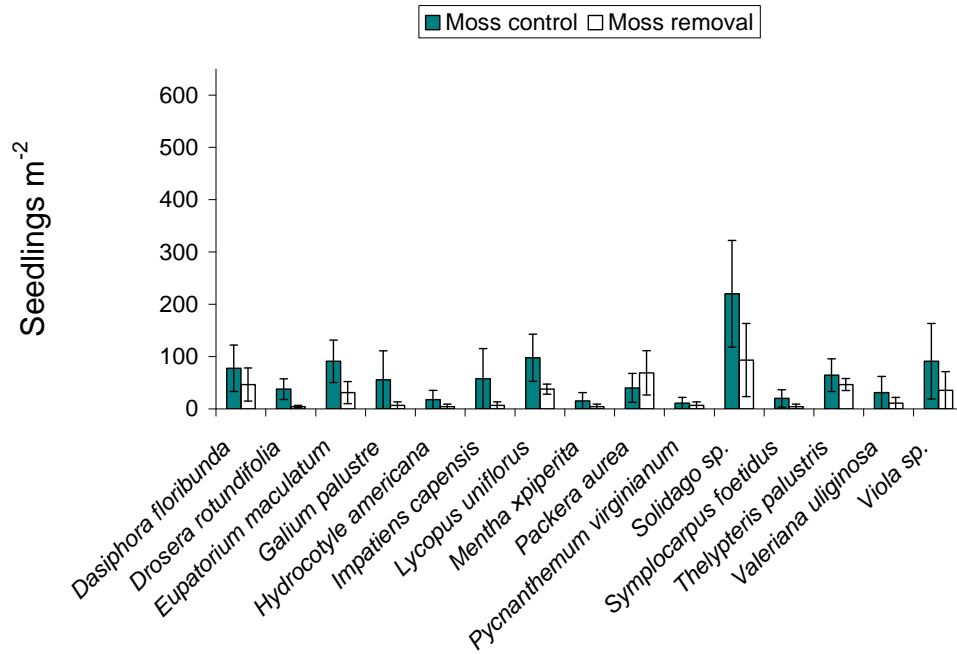


Figure 3-3. Differences in number of forb individuals between (a) areas with mosses present vs. naturally absent at four fens ($p < 0.001$ for height < 5 cm; $p < 0.05$ for height > 5 cm), and (b) moss control vs. moss removal plots at four fens ($p < 0.0001$ for both height classes). BSF = Belle School Fen, CSE = Clark Street Extension Fen, FF = Fish Fen, LF = Larry's Fen, MRFS = Mott Road Fen South. Error bars \pm SE, $n = 5$.

(a) 2006



(b) 2007

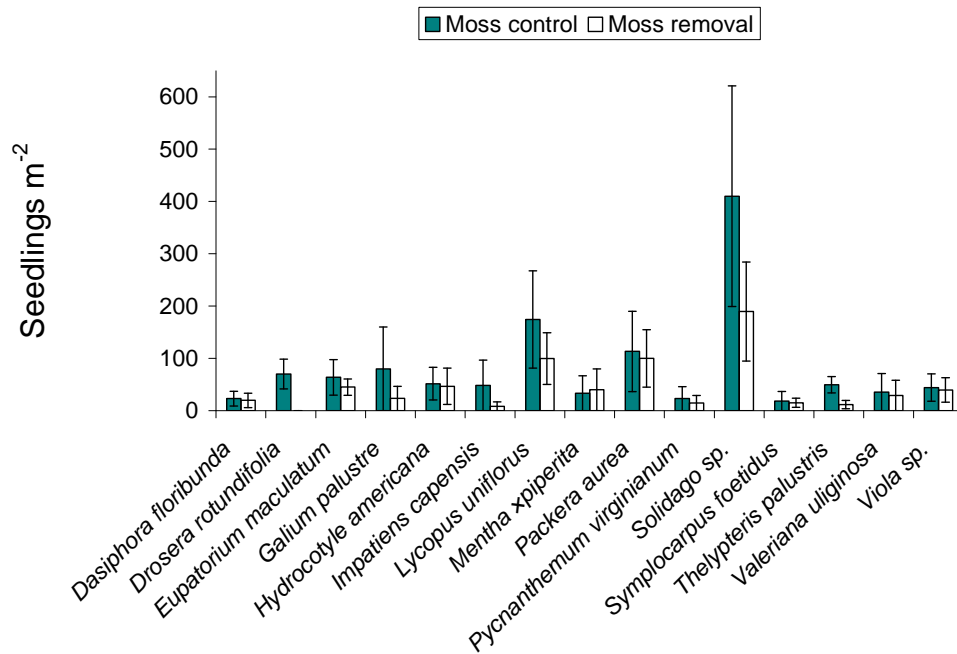


Figure 3-4. Effects of moss removal on the number of forb individuals by species, averaged across rich fen sites. Data are from (a) 2006 for FF, LF, and MRFS and (b) 2007 from CSE, FF, LF, and MRFS. Graphs depict the 15 most abundant forb species. CSE = Clark Street Extension Fen, FF = Fish Fen, LF = Larry's Fen, MRFS = Mott Road Fen South. Error bars +/- SE.

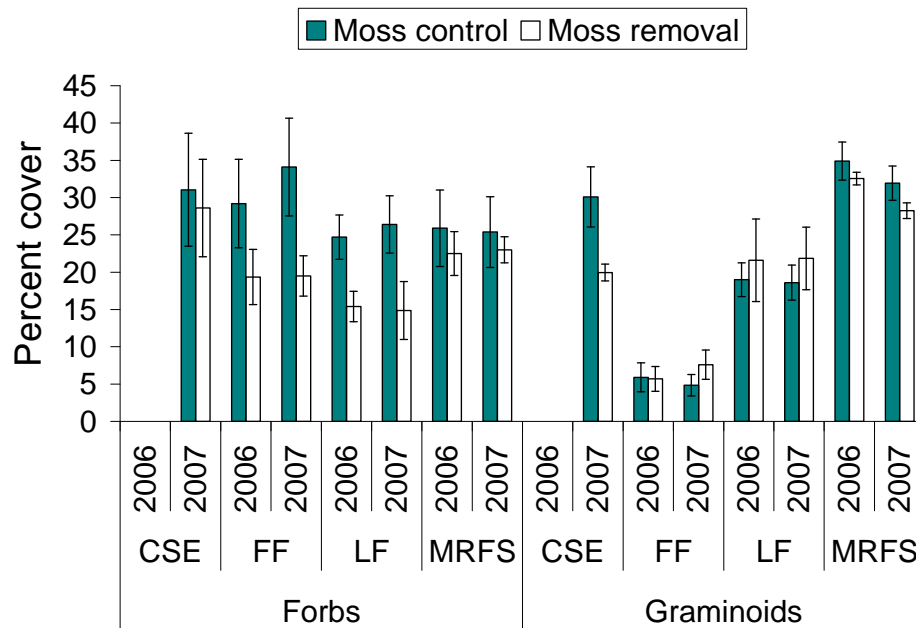


Figure 3-5. Effects of moss removal on total percent cover for forb species ($p < 0.001$ for FF, LF, and MRFS across years; $p < 0.05$ for 2007 alone) and graminoid species (n.s.) in 0.25-m^2 plots. Note that the apparent 2007 difference in graminoid cover at CSE reflects a difference that also was present in 2006, before the moss removal treatment was applied. CSE = Clark Street Extension Fen, FF = Fish Fen, LF = Larry's Fen, MRFS = Mott Road Fen South. Error bars \pm SE, $n=5$.

count data was significantly greater at all sites where mosses occurred than where they were absent, both in the field survey and in the moss removal experiment (Figure 3-6). Correspondingly, forb species density for the 0.25-m² moss control and moss removal plots also was greater where mosses occurred for three of the four sites (FF, LF, and MRFS; $p < 0.01$). While no difference was observed at the plot scale for the fourth site (CSE), this may be a result of sampling error, as forb species density was higher in moss removal than in moss control plots at CSE before the removal treatment was applied. In contrast to the results for forb species, but corresponding to differences in percent cover estimates, graminoid species density showed no difference between moss control plots and those where mosses had been removed ($p = 0.94$). Similar to the seedling count data, no difference in percent cover or species density was evident between moss control and disturbance control plots established at FF or LF.

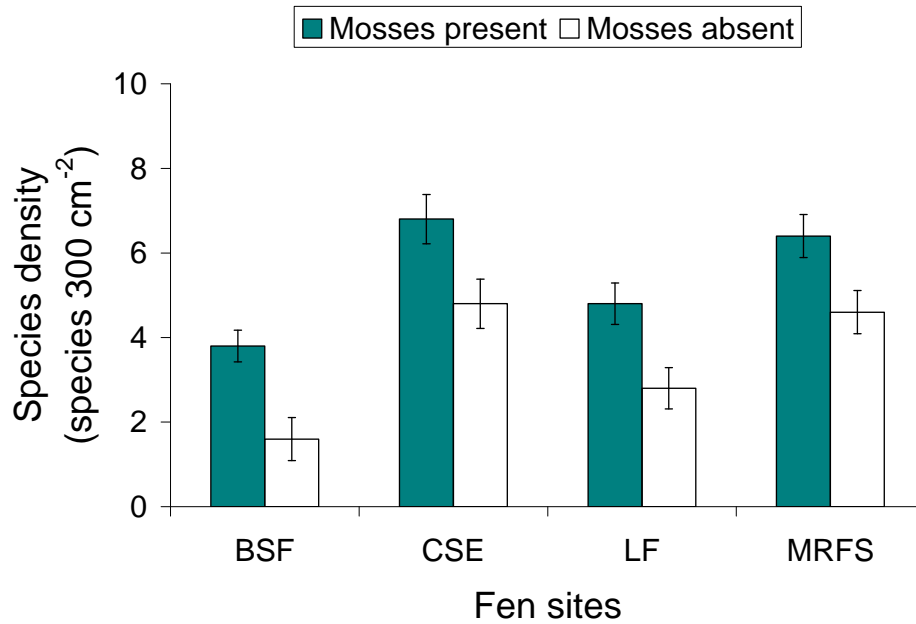
DISCUSSION

Under both field survey and experimental conditions, the presence of mosses appeared to facilitate seedling establishment and increase the abundance and diversity of forb species in rich fens. In contrast, graminoids showed no response to mosses. Such positive interactions between mosses and forb species may help to explain the high forb species densities of New York rich fens. This work also provides additional evidence for the importance of positive plant species interactions in environmentally stressful habitats.

Mosses improved forb seedling emergence and establishment

The presence of mosses resulted in greater seedling emergence for *Solidago patula* (Figure 3-1a), greater seedling survival for *Solidago patula*, *Eupatorium maculatum*, *Packera aurea*, and *Mentha x piperita* (Figures 3-1b and 3-2), and greater

(a) Field survey



(b) Moss removal experiment

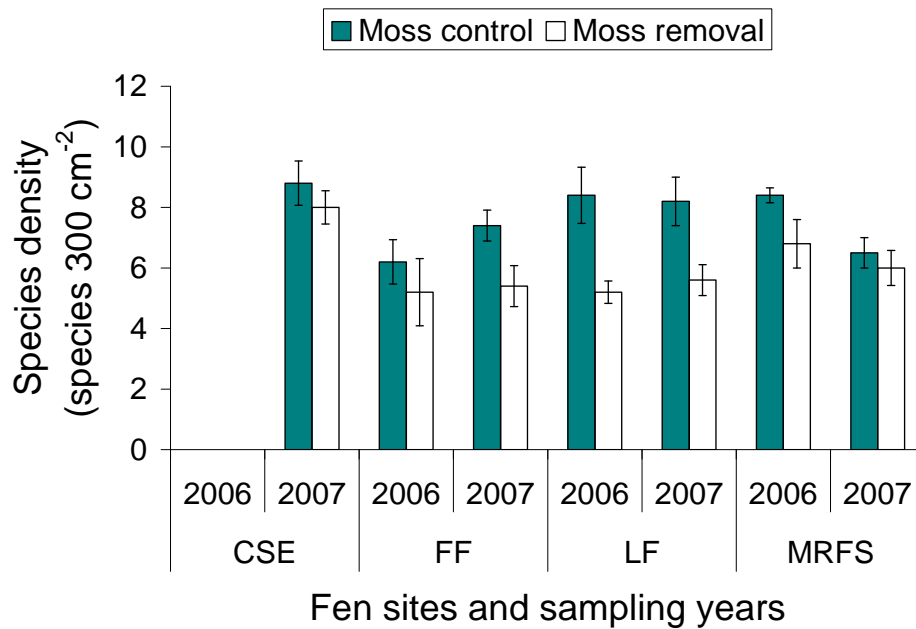


Figure 3-6. Differences in forb species density between (a) areas with mosses present vs. naturally absent at four fens ($p < 0.001$), and (b) moss control vs. moss removal plots at four fens ($p < 0.01$). Species density estimates are based on counts of individual plants in three 100-cm² subplots per plot area. BSF = Belle School Fen, CSE = Clark Street Extension Fen, FF = Fish Fen, LF = Larry's Fen, MRFS = Mott Road Fen South. Error bars \pm SE, $n = 5$.

seedling abundance overall (Figures 3-3 and 3-4). Of the species considered, only seedling emergence for the wetland generalist *Eupatorium maculatum* was reduced where mosses occurred. Effects of mosses on seedling emergence and survival may be positive (e.g., Freestone 2006) or negative, depending on characteristics of the plant species and of the habitat (reviewed in During and van Tooren 1990). In contrast to these results, however, mosses typically have been found to inhibit seedling emergence and survival in herbaceous wetlands (Spackova et al. 1998, Kotorova and Leps 1999, Spackova and Leps 2004, Stammel et al. 2006), although this inhibitory effect may be reduced or removed for species of larger seed size (Kotorova and Leps 1999, Stammel et al. 2006). In a notable exception, seedling survival for *Succisella inflexa* (Kluk) G. Beck (southern succisella), a rare fen species in Germany, was improved where mosses occurred (Overbeck et al. 2003).

A potential explanation for these apparently contradictory results may lie in a shift between positive and negative plant species interactions along environmental stress gradients across herbaceous wetland systems (e.g., Bertness and Callaway 1994, Brooker et al. 2008). While not consistent with most other herbaceous wetland studies, these findings of positive moss effects on seedling emergence and survival are in agreement with studies from some other stressful environments, such as high-arctic meadows (Sohlberg and Bliss 1987) or limestone grasslands (Ryser 1993). Based on his study of seedling establishment in the presence of mosses and vascular plants in limestone grasslands, Ryser (1993) suggested that while competition (with either vascular plants or bryophytes) may determine seedling establishment under high productivity, other factors may gain importance where productivity is reduced. Generalized across systems and species, the stress-gradient hypothesis suggests that positive species interactions may be prevalent under high environmental stress, but shift toward competitive interactions under lower stress (Bertness and Callaway 1994,

Brooker et al. 2008). Such a shift has been demonstrated for interactions between vascular plant species in several environmentally severe systems, such as alpine zones or areas of moisture limitation (Choler et al. 2001, Cavieres et al. 2006), although it may not be general to all cases (Maestre et al. 2005). If mosses in New York and Michigan fens alleviated an environmental stress experienced to a lesser extent (or overwhelmed by competitive interactions) in other herbaceous wetlands, this stress gradient could help explain the range of positive and negative responses to the presence of mosses across different wetland sites.

One likely stress gradient to link contrasting effects of mosses across these different wetlands may be a hydrologic one. Water levels in New York fens tend to remain at or near the peat surface throughout the growing season (Boomer and Bedford 2008b, Chapter 1), and at three New York sites redox potential was greater underlying mosses than where mosses had been removed (Chapter 1). This has implications for emerging seedlings, which tend to be more successful under oxidized conditions and where water levels are below the surface (Fraser and Karnezis 2005); Kercher and Zedler (2004) found fen species to be particularly sensitive to high water levels. While rooted in the peat at maturity, emerging seedlings also are elevated slightly above the peat surface where mosses occur, which would magnify the redox difference. Additionally, concentrations of reduced iron at a depth of 1 cm were greater where mosses had been removed (Chapter 1), in some locations reaching levels shown to inhibit growth of particularly iron-sensitive fen forb species (Snowden and Wheeler 1993), which could make iron toxicity a threat in the absence of mosses. Snowden and Wheeler (1993) found that fen graminoids were least sensitive and forb species most sensitive to iron toxicity, such that some forbs were localized to drier sites with more oxidized soil conditions and lower iron concentrations. Alleviation of reducing conditions in shallow soils thus may be a mechanism by which mosses

improve seedling emergence and survival of rich fen forb species in these New York and Michigan sites.

In contrast, where moss effects on seedling emergence or survival have been studied for other herbaceous wetlands, water levels appear not to have been stressful to seedlings. These studies attribute moss inhibition of seedling emergence and survival instead to competitive interactions, such as direct physical interference, reduction of light availability, or prevention of small seeds (and roots of new seedlings) from reaching the substrate. The only study to propose a hydrologic mechanism also was the only other herbaceous wetland study to show a facilitative effect of mosses: Overbeck et al. (2003) found that mosses improved seedling survival for a rare species in a rich fen in Germany, potentially by protecting seedlings from severe drying of surface soils after annual flooding and drawdown. I propose that the German, New York, and Michigan rich fens represent situations of potentially high hydrologic stress to forb species, compared with other studied wetland sites; hence, under high hydrologic stress, moss facilitation favors seedling emergence and survival, whereas under low stress, competition inhibits them.

A second stress to plants that mosses may help to alleviate is phosphorus limitation. Previous work indicated that uptake of phosphorus by forb seedlings was greater where mosses occurred, and that more oxidized soil conditions were a key driver of greater phosphorus supply (likely through greater microbial mineralization), increased phosphorus acquisition (through greater root phosphatase activity, root colonization by arbuscular mycorrhizal fungi, and root proliferation), and therefore greater phosphorus uptake (Chapter 1, Chapter 2). Thus, improving phosphorus uptake under phosphorus-limited conditions may be an additional mechanism by which mosses facilitate fen forb seedling establishment. While other wetland studies of moss effects on seedling emergence or survival did not address soil nutrient

conditions specifically, it is plausible that greater nutrient availability at those sites than in New York and Michigan fens may have contributed to the competitive interactions between mosses and vascular plants.

Additional mechanisms contributing to the facilitative effect of rich fen mosses on vascular plant species remain possible. Freestone (2006) found a strong positive effect of a moss species on frequency and local abundance of a serpentine seep specialist, but these positive interactions unexpectedly were not stronger at the harsher ends of moisture, toxicity, or biomass gradients. She concluded that the balance between facilitation and competition did not relate to environmental stress in this case, and that seed retention was instead a likely mechanism improving recruitment. Moss seed trapping and retention, protection from seed predation, or protection from seedling herbivory by slugs all have the potential to play a role in moss facilitation in rich fens (During and van Tooren 1990). Retention or protection of seeds by mosses clearly was not necessary in the sites where removal of mosses enhanced seedling emergence, however, suggesting that these mechanisms may not be critical here. Because forb species typical of rich fens often have small, short-lived seeds, annual seed rain is likely more important to seedling emergence than retention in the seed bank.

Mosses promoted forb abundance and diversity

The positive effects of rich fen mosses on seedling emergence and survival were reflected in measures of plant species composition and diversity. In both the field survey and the moss removal experiment, greater forb seedling abundance where mosses occurred was observed both for seedlings under 5 cm in height and individuals over 5 cm (Figure 3-3), suggesting that moss effects on seedling survival were carried forward into the numbers of well-established plants. Additionally, abundance of

individuals was greater or equivalent where mosses occurred for the 15 most abundant species observed in the moss removal experiment (Figure 3-4). While I tracked seedling emergence and survival only for a small subset of fen species, natural establishment rates in moss-covered and moss-free areas under natural and experimental conditions suggest that conclusions drawn from tracked species may be more broadly applicable. Finally, total forb cover and forb species density also were greater where mosses occurred, while cover and species density of graminoids (predominantly sedges) showed no response to mosses (Figures 3-5 and 3-6). While it is possible that effects on forb species density were those to be expected from differences in the total number of individual plants (e.g., Jutila and Grace 2002), observed differential effects of mosses on forbs vs. graminoids would be expected to influence species composition and richness overall. Thus, these results suggest that the positive effects of mosses may help explain the significant contribution of forb species to the high diversity of rich fens in New York and Michigan.

Other investigations of interactions between mosses and vascular plants typically have not found significant effects of mosses on community composition. In an oligotrophic meadow in the Czech Republic, Spackova and Leps (2004) found that while seedling abundance was greater and seedling species composition altered where they removed mosses, moss removal did not affect the species composition of the established vegetation after four years. Similarly, in a study of arctic tundra where seed germination was greater following moss removal, mosses had no effect on the survival of adult transplants (Gough 2006). The magnitude of moss effect at the community level may have been low in these studies because a negative effect of mosses on mature plants would only become evident over long time periods. In contrast, in New York and Michigan fens, seedlings in the absence of mosses were

less likely to survive at all; thus, the effects of mosses on community composition and diversity were measurable more quickly.

These positive effects of mosses on rich fen forb species density support recent models incorporating positive plant species interactions into Grime's hump-backed diversity curve (Hacker and Gaines 1997, Michalet et al. 2006). Hacker and Gaines (1997) initially suggested that positive interactions between plant species might increase diversity by aiding species that otherwise might not survive under conditions of high disturbance, stress, or predation. In rich fens, the most likely beneficiaries under this model might be the rare, uncommon, or specialist forb species that comprise a significant portion of the diversity of these systems. This study did not explore the response to mosses of rare fen species because such species occurred in field survey and experimental plots in numbers too low to analyze statistically. I did, however, investigate seedling survival for two fen specialists, *Solidago patula* and *Packera aurea*, both of which demonstrated significantly greater survival where mosses occurred (Figures 3-1b and 3-2a).

Michalet et al. (2006) noted that the Hacker and Gaines (1997) model implies a continuous increase in diversity (through facilitation) to very high levels of environmental severity, without explaining the decrease in species density generally observed at the highest levels of environmental stress. Michalet et al. (2006) therefore suggested that the model be modified to address declines in diversity at very high as well as very low levels of environmental stress. In their revision of the model, at the highest levels of environmental stress only a small number of species can persist, which would explain the reduction in species density in this portion of the hump-backed curve. At moderate to high levels of stress, however, such stress-tolerant species (through facilitation) could expand the ecological niches of stress-intolerant species (Hacker and Gaines 1997, Choler et al. 2001, Bruno et al. 2003)—thus

increasing overall species densities in areas of moderate to high environmental stress (Michalet et al. 2006). In the context of moss effects on species density in rich fens, this model implies that the highest forb densities would occur where mosses facilitate seedling emergence and survival of wetland generalist species, enabling them to persist in an otherwise harsh fen environment.

This is a novel way of considering species diversity in rich fens, where efforts to explain diversity frequently focus on the rare or specialist species rather than the common components of the wetland flora. Bedford and Godwin (2003) observed, however, that Midwestern temperate fens contain many wetland generalist species in addition to a smaller number of fen specialists. In this study, I investigated seedling survival for two diagnostic rich fen species of New York (*Solidago patula* and *Packera aurea*) (Reschke 1990), a wetland generalist species (*Eupatorium maculatum*), and a weedy hybrid of two introduced species (*Mentha x piperita*). Both where mosses occurred and where they were absent, the fen specialist species demonstrated greater survival than did the generalists by the end of the growing season (Figures 3-1b and 3-2a). For all four species, however, seedling survival was greater where mosses were present (Figures 3-1b and 3-2a); this was true even for *Eupatorium maculatum*, which had shown lower seedling emergence where mosses occurred. These results suggest that moss facilitation of seedling survival may both help fen specialists to thrive, and allow generalist species to become established in rich fens, in areas where stressful environmental conditions otherwise might preclude seedling establishment. While the generalists may not be as successful initially as the more stress-tolerant rich fen specialists, they may become established with help, thus enabling them to contribute to the high species richness of these systems. The Michalet et al. (2006) integration of recent work on facilitation into the hump-backed

model of species diversity may indeed help explain patterns of high species richness in rich fens of the Great Lakes region.

Summary and conclusions

Increasingly, mosses are recognized as important drivers of plant species dynamics and biogeochemical processes in upland and aquatic ecosystems (van Breemen 1995, Vitt 2000, Malmer et al. 2003, Sedia and Ehrenfeld 2003, Nilsson and Wardle 2005, Gornall et al. 2007). This work suggests that in rich fens as well, this previously overlooked plant group is critical in helping to structure the vascular plant community. Mosses in New York and Michigan rich fens had positive effects on forb seedling emergence, survival, and establishment, and these effects on survival of young plants were evident in greater total forb cover and species density where mosses occurred. In contrast, the presence of mosses had no effect on graminoid cover or species density. The presence of mosses improved survival for wetland generalist as well as specialist species, suggesting that mosses may contribute to high forb diversity in rich fens by enabling wetland generalists to become established under conditions of moderate to high environmental stress (Michalet et al. 2006).

Previous work showed that rich fen mosses increased redox potential in shallow soils and improved plant phosphorus uptake in a phosphorus-limited system (Chapter 1, Chapter 2). Given the sensitivity of seedlings to high water levels and to nutrient limitation, it is possible that moss facilitation of forb establishment occurs through alleviation of these dual environmental constraints. Characterization of hydrologic conditions and nutrient availability in wetlands where the effects of mosses on seedling establishment are known could clarify the extent to which these gradients drive a shift between facilitative and competitive interactions. Interactions between mosses and vascular plants in wetlands thus may provide additional insight into the

broad applicability of the stress-gradient hypothesis at the community level (Brooker et al. 2008).

Understanding the facilitative role of mosses in rich fens has important implications for conservation and restoration. In many cases, restoration of aquatic systems may be more effective when positive plant species interactions are incorporated into restoration strategies (Halpern et al. 2007). For rich fens, as in other peatland systems (Rochefort 2000), effectively establishing mosses may be an essential first step to successful restoration of a diverse plant community.

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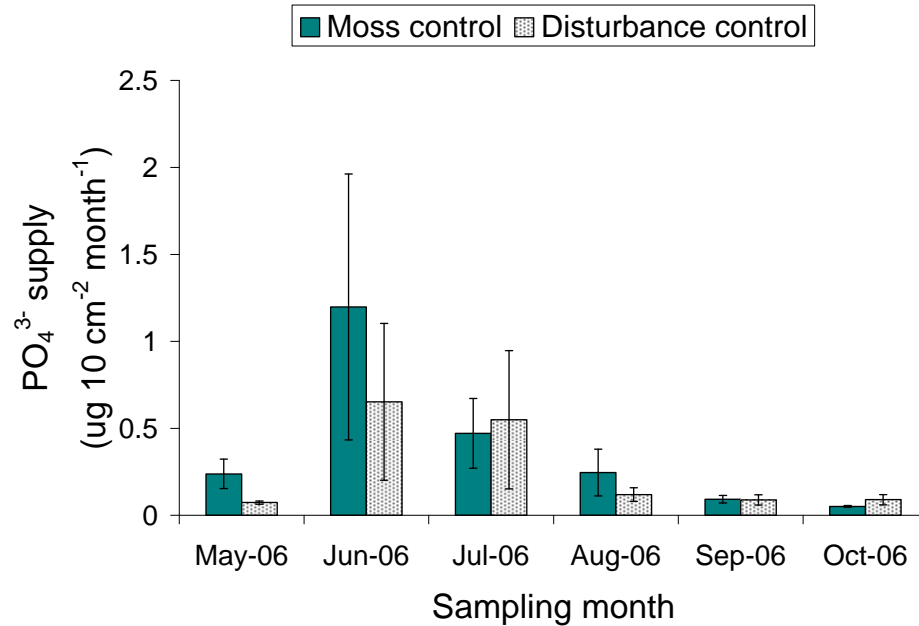
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APPENDIX

Comparison of phosphate (PO_4^{3-}) supply to anion exchange membrane (AEM) resin strips between moss control and disturbance control plots in 2006 for (a) Fish Fen (FF) and (b) Larry's Fen (LF). Across the growing season, PO_4^{3-} supply differed significantly between moss control and disturbance control plots at FF ($p=0.037$, indicating a need for further equilibration) but not at LF (n.s.). Error bars \pm SE, $n=5$.

(a) FF



(b) LF

