Biogeochemistry (2014) 120:203–224
DOI 10.1007/s10533-014-9991-0

Ecoenzymatic stoichiometry and microbial processing of organic matter in northern bogs and fens reveals a common P-limitation between peatland types

Brian H. Hill · Colleen M. Elonen · Terri M. Jicha · Randall K. Kolka · LaRae L. P. Lehto · Stephen D. Sebestyen · Lindsey R. Seifert-Monson

Received: 29 January 2014 / Accepted: 23 April 2014 / Published online: 27 May 2014

Abstract We compared carbon (C), nitrogen (N), and phosphorus (P) concentrations in atmospheric deposition, runoff, and soils with microbial respiration [dehydrogenase (DHA)] and ecoenzyme activity (EEA) in an ombrotrophic bog and a minerotrophic fen to investigate the environmental drivers of biogeochemical cycling in peatlands at the Marcell Experimental Forest in northern Minnesota, USA. Ecoenzymatic stoichiometry was used to construct models for C use efficiency (CUE) and decomposition (M), and these were used to model respiration (R_m). Our goals were to determine the relative C, N, and P limitations on microbial processes and organic matter decomposition, and to identify environmental constraints on ecoenzymatic processes. Mean annual water, C, and P yields were greater in the fen, while N yields were similar in both the bog and fen. Soil chemistry differed between the bog and fen, and both watersheds exhibited significant differences among soil horizons. DHA and EEA differed by watersheds and soil horizons, CUE, M, and R_m differed only by soil horizons. C, N, or P limitations indicated by EEA stoichiometry were confirmed with orthogonal regressions of ecoenzyme pairs and enzyme vector analyses, and indicated greater N and P limitation in the bog than in the fen, with an overall tendency toward P-limitation in both the bog and fen. Ecoenzymatic stoichiometry, microbial respiration, and organic matter decomposition were responsive to resource availability and the environmental drivers of microbial metabolism, including those related to global climate changes.

Keywords Decomposition · Ecoenzymes · Peat · Soil · Stoichiometry

B. H. Hill (✉) · C. M. Elonen · T. M. Jicha · L. L. P. Lehto
Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, US Environmental Protection Agency, 6201 Congdon Blvd., Duluth, MN 55804, USA
e-mail: hill.brian@epa.gov

R. K. Kolka · S. D. Sebestyen
Center for Research on Ecosystem Change, US Forest Service, Northern Research Station, Grand Rapids, MN 55744, USA

L. R. Seifert-Monson
Integrated Biosciences Graduate Program, University of Minnesota, Duluth, MN 55812, USA

Present Address:
L. R. Seifert-Monson
Sappi Fine Paper North America, Ltd., Cloquet, MN 55720, USA

Introduction

Peatlands are a significant component in global carbon (C) budget because of the quantity of C stored in their soils and vegetation, and because of their uptake and release of the greenhouse gases, carbon dioxide (CO_2),

methane (CH_4), and nitrous oxide (N_2O). Globally, wetland C storage is estimated to be 529 Pg (Pg = 10^{15} g), of which 462 Pg is stored in peatlands (370 Pg C as peat; Bridgham et al. 2006; Kayranli et al. 2010). Wetlands of the conterminous US store 20 Pg C of which 14 Pg C is in peatlands, though annual peatland C uptake by primary production is nearly offset by respiratory C losses, resulting in small incremental increases in peatland C mass (Bridgham et al. 2006; Limpens et al. 2008). As such, the role of peatlands in ameliorating rising CO_2 in the atmosphere lies both in maintaining their large C stores as well as their potential to accumulate new C. However, this peatland C pool, under the influence of warmer temperature and lowered water tables, could potentially release large quantities of CO_2 under aerobic conditions, and CH_4 and N_2O, as a result of anaerobic respiration (Bridgham et al. 2006; Limpens et al. 2008; Kayranli et al. 2010).

The amount of C sequestration in peatlands may depend on nitrogen (N) and phosphorus (P) limitations on primary productivity (Bridgham et al. 1996, 1998). Atmospheric deposition is the primary N input to most peatlands (Urban et al. 2011), and the fate of this N depends on attributes of the watersheds (Alexander et al. 2009). Watersheds that have been minimally disturbed by anthropogenic activities, are well-buffered by calcium (Ca) and magnesium (Mg), and have low N storage, will have greater capacity to assimilate N inputs than those watersheds lacking these attributes (Hedin et al. 1995). As N deposition on a watershed approaches the threshold for N assimilation (i.e., a critical N load), the ability to retain N decreases, resulting in increased N in runoff primarily as NO_3^- and dissolved organic N (Stoddard 1994).

Northern peatlands are particularly sensitive to N additions, owing to their unique hydrological and biogeochemical properties, and these peatlands are divided into two broad classes on the basis of pH and hydrology (Gorham et al. 1985; Urban et al. 2011). Minerotrophic fens have precipitation, groundwater, and surface water inputs and are slightly acidic to neutral pH, while ombrotrophic bogs receive water and nutrients from atmospheric deposition and are acidic. Northern fens and bogs are dominated by *Sphagnum* mosses (*Sphagnum* spp.) at the surface, with fens having much more diverse plant communities than bogs. Fens range from open to forested (e.g., white cedar, *Thuja occidentalis*; black spruce, *Picea mariana*; or eastern larch, *Larix laricina*) while bogs are generally forested by black spruce (Bridgham et al. 1996). Both bogs and fens have distinct vertical soil zonation, with actively photosynthesizing vegetation above a peat zone that is transiently aerobic or anaerobic depending on water table elevation (the acrotelm), which is underlain by a layer of peat below which the water table infrequently drops and is consequently almost always anaerobic (the catotelm; Limpens et al. 2008). Bogs in ice-block depressions have an added topographic feature, the lagg, at the interface with the toe of the upland slope (Verry et al. 2011a). The lagg has been identified as a biogeochemical "hot spot," a zone of particularly high biogeochemical activity (Mitchell et al. 2008).

The accretion and decay of C is stoichiometrically related to N and P availability such that increases in primary production (C accretion) and heterotrophic respiration (C decay) are often positively correlated with N and P availability (Bridgham et al. 1996; Keller et al. 2006). While both N and P co-vary with C in peatlands, it appears that P availability is more likely limiting for peatland biogeochemical processes than either C or N (Bridgham et al. 1996; Kellogg and Bridgham 2003; Seifert-Monson 2013). P additions to bogs originate from atmospheric deposition (Verry and Timmons 1977; Bridgham et al. 1996). In addition to C storage, peatlands also store large quantities of organic N and P. Verry and Timmons (1982) reported that 56 % of total N (TN; 80–90 % of labile N) and 76 % of total P (TP) inputs to a peatland were retained rather than exported. However, Bridgham et al. (1998) reported that, while N and P stores in peatlands were large, the available fractions were much smaller and tightly cycled, leading to relative N and P limitations on productivity.

By the above accounting, nearly 50 % of N and 25 % of P inputs to peatlands are exported, mostly as organic forms. Some losses also occur as denitrification or dissimilatory NO_3^- reduction to NH_4^+, though denitrification accounts for less than 5 % of NO_3^- removal from bogs (Urban et al. 1988; Keller and Bridgham 2007). Those authors attributed low denitrification rates to low NO_3^- availability and low pH, both of which have been found to be limiting to denitrification and dissimilatory NO_3^- reduction to NH_4^+ (Hayden and Ross 2005; Keller and Bridgham

2007). Finally, climate change is expected to dramatically alter current C, N, and P dynamics of peatlands. Many authors have hypothesized that increasing temperatures, decreasing water tables, and additions of N will stimulate soil C decomposition, further releasing the greenhouse gases CO_2 and CH_4 (Gorham 1991; Freeman et al. 2004; Keller and Bridgham 2007; Bridgham et al. 2008).

Cellular respiration is the primary pathway for organic C mineralization. Whether or not the respiration occurs in the presence of O_2, the same electron transport system (ETS) supports the generation and transfer of electrons (e^-) as the C-containing compound is degraded and energy released (Broberg 1985). Respiration associated with peat may be measured as ETS activity using dehydrogenase (DHA) enzymes (Trevors et al. 1982; Broberg 1985). Measurement of DHA is based on intercepting e^- flow through mitochondrial and microsomal ETS using a surrogate electron acceptor, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT), which has a redox potential slightly higher than that of the cytochrome electron acceptors used by prokaryotic organisms (Broberg 1985). While anaerobic organisms use different e^- acceptors than O_2 (e.g., NO_3^-, SO_4^{2-}) and have different respiratory enzymes, DHA measurement is applicable to anaerobic ETS activity (Trevors et al. 1982).

In addition to cellular respiration, microbes produce ecoenzymes to catalyze the degradation of organic matter to acquire organically-bound C and nutrients (Sinsabaugh and Foreman 2001; Hill et al. 2006; Sinsabaugh et al. 2009). We use the term ecoenzyme to broadly encompass all enzymes located outside the confines of intact cell membranes regardless of whether such enzymes enter the environment by secretion or lysis (Sinsabaugh et al. 2009). This definition provides the closest correspondence between environmental enzyme activity and organic matter decomposition. Of particular interest are glycosidases used in the processing of labile organic C, glucosaminidases and peptidases used in chitin and protein degradation to acquire N, and phosphatases related to P acquisition. Ecoenzyme activity (EEA) is positively related to sediment nutrient concentrations (Sinsabaugh et al. 2009), organic C refractivity (Sinsabaugh and Follstad Shah 2011), and microbial metabolism (Sinsabaugh et al. 2012).

The stoichiometry of EEA is indicative of the relative nutrient limitations on microbial assemblages and their environments. Organic C sequestration by biota is governed not only by organic C availability, but also by the availability of N and P. The ratio of these components in algae, for example, is expected to be 108C/16N/1P (Redfield 1958). Similarly, the C/N/P of microbial biomass is expected to be 60/7/1 (Cleveland and Liptzin 2007). Departures from these ratios suggest that metabolism and growth of an organism will be limited by the element in short supply and that this limitation will aggregate from organisms to populations and ecosystems (Sinsabaugh et al. 2009).

Microbial C use efficiency (CUE) is a measure of the relative apportionment of C utilization for microbial growth versus the energetic requirements (as respiration) to support existing microbial biomass (Keiblinger et al. 2010; Manzoni et al. 2012; Sinsabaugh et al. 2013). Microbial CUE, which ranges from 0 to 0.60, decreases with increasing temperature, though the response is weak within the temperature range of most studies (Manzoni et al. 2012). Resource C/N/P stoichiometry has proven to be a more important regulator of CUE, with increasing C and decreasing N and P availability resulting in declining CUE (Manzoni et al. 2012; Sinsabaugh et al. 2013).

Our objectives for this study were (1) to investigate the role of microbial ecoenzymes in facilitating organic matter processing in northern peatlands, (2) to determine the relative C, N, and P limitations on microbial processes and organic matter decomposition, and (3) to consider the environmental constraints on microbial EEA processes. We then used enzyme decomposition models, which have not previously been used to study peatland organic matter processing, to explore potential climate change impacts on organic matter decomposition in peatlands.

Methods

Study sites

We studied two peatland watersheds within the US Department of Agriculture's Forest Service Marcell Experimental Forest (MEF; N 47° 30.17′, W 93°28.97′), located approximately 40 km north of Grand Rapids, Minnesota, USA. The MEF is within

the Laurentian Mixed Forest Province, which is a transitional zone between boreal forests to the north and broadleaf deciduous forests to the south (Verry et al. 2011b). The landscape is a typical low-relief, moraine landscape of the Upper Great Lakes Region, and includes uplands, peatlands, and lakes. Peatlands at the MEF range in size from several hectares to several tens of hectares and may have forest, shrub, or sedge cover. The MEF has an extensive historical database of hydrology, chemistry, and meteorology that document hydrological, biogeochemical, and ecosystem processes since the early 1960s (Sebestyen et al. 2011). The climate is sub-humid continental, with wide and rapid diurnal and seasonal temperature fluctuations. Over the period of record (1961–2009), the average annual air temperature was 3 °C, with daily mean extremes of −45 and 38 °C, and the average annual precipitation was 780 mm, most of which falls as rain from mid-April to early November. Mean annual air temperatures have increased about 0.4 °C per decade over the last 40 years (Sebestyen et al. 2011).

Our two study peatlands include an ombrotrophic bog and a minerotrophic fen. The two peatlands are within several km of one another and have similar depositional settings: no dominant aspect, <20 m of topographic relief, and mixed conifer-deciduous forest cover. The bog watershed contains a 3.2 ha bog (*P. mariana*, *Sphagnum* sp.) and a 6.2 ha upland (quaking aspen, *Populus tremuloides*; paper birch, *Betula papyrifera*). Stream pH at the watershed outlet averages 4.1. The bog watershed is instrumented for measurement of precipitation amount, surface and sub-surface runoff, bog water level, and depth to the regional water table, and serves as the undisturbed reference watershed for the southern MEF study unit (Sebestyen et al. 2011). Atmospheric C, N and P inputs to the study bog and fen were based on samples collected from the bog watershed.

The fen watershed has an 18.6 ha fen (*Sphagnum* sp.; willow, *Salix* sp.; *A. rugosa*, *P. mariana*, *T. occidentalis*) surrounded by a 53.4 ha upland (*P. tremuloides*; *B. papyrifera*; balsam fir, *Abies balsamea*; jack pine, *Pinus banksiana*; red pine, *Pinus resinosa*). Stream pH at the outlet averages 6.9. The fen is instrumented for measurement of precipitation amount, bog water level, and depth to the regional water table. The entire fen (but not the uplands) was clear-cut and the slash was burned in 1972–1973 (Sebestyen et al. 2011). Water yields did not change after harvesting and nutrient concentrations had returned to pre-harvest levels by 1976 (Sebestyen and Verry 2011).

Atmospheric deposition and outflow water chemistry

Precipitation event samples were analyzed for total organic C (TOC; Shimadzu TOC-VCP analyzer), nitrate, ammonium, and TP. These values were aggregated to monthly values for this study. Outflow water samples from the bog and fen were collected as a surface grab sample on each of the May–October sampling dates (bog $n = 17$, fen $n = 11$) over the 2010–2012 study. We analyzed each sample for chloride (Cl^-), dissolved (DOC) and TOC, NO_3^-, TP, and SO_4^{2-} concentration. Water subsamples for Cl^-, DOC, NO_3^-, and SO_4^{2-} were filtered through a 0.45 μ pore membrane and preserved according to the analyte within 24 h of collection. Both filtered and unfiltered subsamples for N and P species were preserved frozen until analyzed. All nutrient samples were analyzed using a Lachat flow-injection analyzer. The unfiltered TP subsamples were digested using the persulfate method, and analyzed using the molybdate-ascorbic acid protocol (American Public Health Association, APHA 1998). Nitrate was analyzed using the cadmium reduction method (APHA 1998). Total and DOC samples were preserved with H_3PO_4 and measured by UV-$Na_2S_2O_8$ oxidation and non-dispersive infrared detection with a Dohrmann Phoenix 8000 TOC analyzer (APHA 1998). Water subsamples for SO_4^{2-} and Cl^- were kept cold at 4 °C until analyzed by a Dionex ion chromatograph DX300 (APHA 1998).

Soil collection and chemistry

Upland soil samples were collected with either Plexiglas tube corer or a shovel, and peat samples were collected using a Russian-style peat corer. Soil samples from the bog and uplands were collected once each in May, July and October 2010; May–June, August and October 2011; and in May, July and October 2012. Similar core samples from the fen and uplands were collected twice monthly in May, August and October 2011; and twice monthly in June, August and October 2012. On each sampling date, triplicate soil samples (corer: 3.8 cm diameter, 30 cm depth;

shovel: 100 cm^2, 30 cm depth) from the upland sites were divided into O, A, and B soil horizons. Triplicate cores (5 cm diameter, 60 cm depth) were collected each from the bog (lagg, hummocks, and hollows) and fen (transitional zone, hummocks, and hollows). These cores were divided into the actively growing surface, acrotelm, and catotelm horizons.

We analyzed soil samples for pH, available ammonium (NH_4^+), NO_3^-, total C (TC), TN, and TP. pH was measured directly with an IQ 150 ion sensitive field effect transistor probe. All nutrient extracts/digests were analyzed using a Lachat flow-injection analyzer. Field moist subsamples were extracted with 2 M potassium chloride (KCl) for available NO_3^- and NH_4^+ (Keeney and Nelson 1986); extracts were analyzed using the cadmium reduction and phenolate method (Methods 12-107-04-1E and 12-107-06-1B; Lachat Instruments 2009), respectively. Soil water content was determined by gravimetric methods using a drying oven at 60 °C for 24 h to a constant weight. The percent solids were used to calculate available nutrient content on a dry weight basis. Soil samples were dried and ground for TC, TN, and TP analyses. TC and TN were analyzed by combustion using a Model 1112 EA Carla Erba elemental analyzer; TP was determined by first digesting the sample in reagent grade concentrated nitric acid (HNO_3) using a CEM Corporation microwave, then neutralized with sodium hydroxide (NaOH) and analyzed by the molybdate-ascorbic acid method (Method 10-107-04-1: Lachat Instruments 2009). Soil bulk density (BD, g cm^{-3}) was calculated as the wet mass of the sample divided by the wet volume of the sample (Arshad et al. 1996).

Microbial respiration and ecoenzyme activity

We analyzed each horizon of the upland, bog, and fen soil samples for microbial respiration (DHA) and EEA related to C, N, and P acquisition. Duplicate DHA aliquots (1 g wet weight) were mixed with 2.5 mL of sodium bicarbonate ($NaHCO_3$, pH 8.1) buffer and 1 mL of 0.75 % INT standard, sealed, agitated, and incubated (dark, 27 °C) for 1.5 h. Aliquots were centrifuged (2,000 × *g*) for 5 min and the supernatant analyzed for absorbance (428 nm) using a Model 20 Perkin Elmer UV spectrophotometer. Aliquot absorbance was compared to a standard INT curve (prepared for each sample batch) and normalized by soil C content to calculate DHA activity (nmol INT g^{-1} C h^{-1}). On a molar basis, 2 mol of INT are equivalent to 1 mol of CO_2 respired (Broberg 1985).

C acquisition was measured as β-D-glucosidase (BG; Enzyme Commission [EC] No. 3.2.1.21) activity, N acquisition was measured as the combined activities of β-*N*-acetylglucosaminidase (NAG; EC 3.2.1.50) and L-leucine aminopeptidase (LAP; EC 3.4.11.1), and P acquisition was measured as acid phosphatase (AP; EC 3.1.3.2) activity. These EEA assays used substrates linked to methyl-umbelliferyl (MUB) or methyl-coumarin (MCM) residues (Sigma-Aldrich Corporation, St. Louis, MO, USA). Polyphenol oxidases (POXs, EC 1.14.18.1), a composite of enzymes used to decompose phenolic compounds including lignin, was analyzed using L-3,4-dihydroxyphenylalanine in an acetate buffer.

All EEA assays used the microplate protocols originally developed by Sinsabaugh and colleagues (Sinsabaugh et al. 1997; Foreman et al. 1998; Sinsabaugh and Foreman 2001), but with modifications (Hill et al. 2006). All substrate and reference solutions were prepared in sterile deionized water and included quadruplicate assays for each enzyme and reference standards. Quenching, the decrease of fluorescent emissions caused by the interactions of enzyme substrates with non-reactant chemicals in the assays, was estimated by comparing the fluorescence of the supernatant of standards mixed with soil slurries to that of the standard solution mixed with buffer (German et al. 2011). We incubated the microplates for various times at 22 °C for MUB-linked substrates, and at 30 °C for MCM-linked substrates. Fluorescence was measured using a Model FLX800T BioTek Instruments fluorometer with an excitation wavelength of 350 nm and an emission wavelength of 450 nm. We report EEA as substrate accumulated per unit soil over time, and results are adjusted for emission coefficients calculated from standards, corrected for quenching, and normalized for sample organic C content (nmol g^{-1} C h^{-1}; German et al. 2011).

We used EEA and stoichiometry to determine C quality, nutrient limitation, and CUE (Sinsabaugh et al. 2013) for each sample analyzed. Substrate C quality was estimated using an enzyme-based lignocelluloses index (LCI; Sinsabaugh and Follstad Shah 2011):

$$\mathrm{LCI} = \ln\mathrm{POX}/(\ln \mathrm{BG} + \ln \mathrm{POX}). \quad (1)$$

Because the soil C pool was expected to become more recalcitrant as organic matter decomposition progressed, which would result in slowed microbial growth, EEA stoichiometric ratios were normalized for POX activity to account for the decrease in the demand for P relative to that for N (Sinsabaugh and Follstad Shah 2012). Regression of POX-normalized EEA more accurately reflects relative C, N, and P limitation along the decomposition gradient:

$$\ln \mathrm{BG}/\ln \mathrm{POX} = \ln[\mathrm{NAG} + \mathrm{LAP}]/\ln \mathrm{POX}, \quad (2)$$

$$\ln \mathrm{BG}/\ln \mathrm{POX} = \ln \mathrm{AP}/\ln \mathrm{POX}, \quad (3)$$

with deviations from a slope of 1 indicating relative N (Eq. 2) or P (Eq. 3) limitation.

Nutrient limitation was also measured using vector analysis (length, L; angle, A) of EEA stoichiometry (Moorhead et al. 2013):

$$\text{Vector L} = \sqrt{(\ln \mathrm{BG}/\ln[\mathrm{NAG} + \mathrm{LAP}])^2 + (\ln \mathrm{BG}/\ln \mathrm{AP})^2}, \quad (4)$$

$$\text{Vector A} = tan((\ln \mathrm{BG}/\ln \mathrm{AP}), (\ln \mathrm{BG}/\ln[\mathrm{NAG} + \mathrm{LAP}]))*180/3.1416, \quad (5)$$

with relatively longer vector L indicating greater C-limitation and positive and negative vector A indicating relative degrees of N- and P-limitation, respectively.

CUE was predicted as a saturating function based on N and P supply relative to C availability (Sinsabaugh et al. 2013):

$$\mathrm{CUE} = 0.6^{*}\left(\mathrm{CN}_{\mathrm{S}}^{*}\mathrm{CP}_{\mathrm{S}}\right)/((\mathrm{K}_{\mathrm{CN}} + \mathrm{CN}_{\mathrm{S}})^{*}(\mathrm{K}_{\mathrm{CP}} + \mathrm{CP}_{\mathrm{S}}))^{0.5}, \quad (6)$$

where CN_{S} is the C/N of microbial biomass relative to available C/N [8.6/(TC/TN)], CP_{S} is the C/P of microbial biomass relative to available C/P [60/(TC/TP)], and K_{CN} and K_{CP} are the half-saturation constants (0.5).

Organic C decomposition (M, % day^{-1}) was modeled using a microbial ecoenzyme allocation model (Sinsabaugh and Moorhead 1994). We substituted CUE, as calculated by Sinsabaugh et al. (Eq. 6), for the static enzyme degradation efficiency ($\mathrm{k_c} = 0.2$; Sinsabaugh and Moorhead 1994):

$$\mathrm{M} = (\mathrm{CUE}^{*}\mathrm{ENZ}_{\mathrm{TOT}})/(1+([\mathrm{NAG} + \mathrm{LAP}]/\mathrm{BG}) + (\mathrm{AP}/\mathrm{BG})), \quad (7)$$

where $\mathrm{ENZ}_{\mathrm{TOT}}$ is the sum of BG, POX, [NAG + LAP], and AP normalized to their maximum values. Normalized values were used for BG, [NAG + LAP], and AP in the remainder of the equation.

Modeled respiration ($\mathrm{R_m}$, mmol C m^{-3} day^{-1}) associated with decomposition was estimated as the product of the organic C pool, M, and the BD of the soil:

$$\mathrm{R_m} = \mathrm{TC}^{*}\mathrm{M}^{*}\mathrm{BD}, \quad (8)$$

where BD is the bulk density of the soil (kg m^3).

Statistical analyses

We compiled descriptive statistics (mean ± standard error of the mean) for soil C, N and P concentrations, microbial DHA, and microbial EEA related to C, N, and P acquisition. We tested for differences within the bog (lagg, hummocks, hollows) and fen (transitional, hummocks, hollows), and for differences between the bog versus the fen watersheds, uplands versus the bog and fen, seasons, soil horizons, and their interactions, using a Type III General Linear Model of an unbalanced, nested sampling design. We evaluated the relationships between soil chemistry and microbial activity using Spearman rank correlation (r) to avoid problems associated with non-normal data distribution. The stoichiometric relationships of C, N, and P acquiring EEA, and the relationships between CUE, M, and DHA, were modeled using orthogonal regression analyses. All analyses were done using SAS for Windows, release 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Average atmospheric inputs during the 2010–2012 study were 23.0 kg TOC ha^{-1} year^{-1}, 6.40 kg total inorganic N (TIN) ha^{-1} year^{-1}, and 0.14 kg TP ha^{-1} year^{-1} (Table 1). The two watersheds differed markedly in the BD of their mineral and peat soil horizons. Soils on the bog uplands were denser than those on the fen uplands, reflecting their different origins as glacial till on the bog watershed and glacial outwash on the fen

Table 1 Mean annual precipitation (PPT) and the atmospheric deposition of TOC, TIN and TP on the bog; upland, peatland, and total watershed area; soil bulk density; watershed outflow chemistry during the May–October study periods (2010–2012); and annual yields of water, TOC, TIN, and TP for the bog and the fen at the Marcell Experimental Forest

Atmospheric deposition		
PPT (mm $year^{-1}$)	764	
TOC ($kg\ ha^{-1}\ year^{-1}$)	23.0	
TIN ($kg\ ha^{-1}\ year^{-1}$)	6.40	
TP ($kg\ ha^{-1}\ year^{-1}$)	0.14	
	Bog	Fen
Watershed area		
Peatland (ha)	3.2	18.6
Upland (ha)	6.2	53.4
Total (ha)	9.4	72.0
Upland soil bulk density		
O horizon ($g\ cm^{-3}$)	0.61	0.37
A horizon ($g\ cm^{-3}$)	1.37	0.85
B horizon ($g\ cm^{-3}$)	1.41	0.96
Peat bulk density		
Surface ($g\ cm^{-3}$)	0.07	0.09
Acrotelm ($g\ cm^{-3}$)	0.10	0.31
Catotelm ($g\ cm^{-3}$)	0.13	0.78
Outflow chemistry		
Cl ($mg\ L^{-1}$)	1.23	0.71
DOC ($mg\ L^{-1}$)	71.5	19.4
TOC ($mg\ L^{-1}$)	80.3	19.5
NO_3^- ($\mu g\ L^{-1}$)	711	409
TP ($\mu g\ L^{-1}$)	301	32.3
SO_4^{2-} ($mg\ L^{-1}$)	1.00	0.67
Annual yields		
H_2O yield ($m^3\ ha^{-1}\ year^{-1}$)	1,269	10,453
TOC ($kg\ ha^{-1}\ year^{-1}$)	73.4	160
TN ($kg\ ha^{-1}\ year^{-1}$)	0.70	0.73
TP ($kg\ ha^{-1}\ year^{-1}$)	0.18	0.51

watershed. Peat samples from bog were less dense than those from the fen (Table 1). Mean annual water yield from the bog was 1,269 $m^3\ ha^{-1}\ year^{-1}$, compared to 10,453 $m^3\ ha^{-1}\ year^{-1}$ from the fen. C and nutrient exports from the bog averaged 73.4 kg TOC $ha^{-1}\ year^{-1}$, 0.70 kg TN $ha^{-1}\ year^{-1}$, and 0.18 kg TP $ha^{-1}\ year^{-1}$, respectively, compared to exports of 160 kg TOC $ha^{-1}\ year^{-1}$, 0.73 kg TN $ha^{-1}\ year^{-1}$, and 0.51 kg TP $ha^{-1}\ year^{-1}$ from the fen (Table 1).

Analyses of variance indicated few significant differences in microbial DHA or EEA between soil samples collected within the bog (lagg, hummocks, hollows) or within the fen (transitional, hummocks, hollows). Data from these cores were combined to yield average values for cores from the bog and fen. All data and analyses are presented as comparisons of the bog upland and bog with the fen upland and fen.

There were significant differences in outflow water chemistry between the bog and the fen watersheds, with all measured variables being higher for the bog watershed (Tables 1, 2, 3). There were also significant seasonal differences in Cl^-, DOC, TOC, and SO_4^{2-} (Table 3). Soil chemistry also exhibited a significant watershed effect for all variables except for NO_3^-, and significant soil horizon effect for all variables (Tables 2, 3). There were no seasonal differences in soil chemistry (Table 3). The stoichiometric ratios (C/N, C/P, and N/P) for soil chemistry indicated significant differences (with the exception of C/P) between the bog and the fen watersheds, and between the uplands and the bog/fen in these watersheds (Tables 2, 3). The stoichiometric ratios also exhibited significant seasonal and soil horizon effects (Table 3). The LCI was higher in the fen than in the bog watershed, and increased with lower soil horizons, but there were no seasonal differences in LCI (Tables 2, 3).

Microbial DHA, [NAG + LAP], and POX were not significantly different between the bog and fen watersheds, but significant watershed effects were noted for BG and AP (Tables 4, 5). Seasonal effects were seen only for [NAG + LAP] and AP, whereas soil horizon was a significant factor for all DHA and EEA (Table 5). The relative allocation of EEA toward C acquisition was significantly higher in the bog watershed than in the fen watershed. This greater C-limitation in the bog was also evident by the longer enzyme vector L compared to those for the fen (Tables 4, 5). The opposite was true for P acquisition, though this was not supported by enzyme vector A (Tables 4, 5). There was no watershed effect on N acquisition (Table 5). N and P acquiring EEA, and vector A, exhibited significant seasonal responses. All C, N, and P acquisition, and vectors L and A, were significantly different by soil horizons, with C acquisition and vector L increasing with depth, and N and P acquisition and vector A decreasing with depth

Table 2 Mean soil chemistry, soil stoichiometry, and the lignocelluloses index (LCI) for upland soil horizons from the bog and the fen at the Marcell Experimental Forest

	Bog watershed						Fen watershed					
	Upland			Bog			Upland			Fen		
	O	A	B	SUR	ACR	CAT	O	A	B	SUR	ACR	CAT
Soil chemistry												
pH	5.26	5.04	5.09	4.00	3.80	3.78	4.33	5.04	5.11	4.99	5.06	5.74
NH_4^+ (mg kg^{-1})	4.80	2.35	0.98	29.3	52.5	69.0	3.86	1.02	0.70	20.5	16.2	14.8
NO_3^- (mg kg^{-1})	0.70	0.42	0.12	4.87	3.99	2.43	0.64	0.13	0.15	2.85	1.87	0.93
TC (mg kg^{-1})	92,216	18,756	5,057	474,062	481,869	494,755	147,518	14,463	6,678	425,681	277,668	179,583
TN (mg kg^{-1})	5,178	1,383	1,021	13,192	15,969	17,646	9,450	2,757	3,598	33,802	24,694	15,907
TP (mg kg^{-1})	499	426	437	615	548	670	355	159	585	639	308	218
Soil stoichiometry												
Soil C/N	22.1	21.0	15.7	49.1	37.9	34.0	27.1	23.6	17.2	31.4	27.8	23.7
Soil C/P	480	118	33.6	2,309	2,421	2,327	1,042	302	33.0	2,438	2,252	1,506
Soil N/P	22.8	7.97	8.65	53.5	67.3	70.2	62.6	62.0	16.6	163	161	147
Lignocellulose index												
LCI	0.47	0.53	0.57	0.46	0.49	0.52	0.51	0.57	0.60	0.47	0.49	0.54

Samples were collected May–October 2010–2012

(Table 5). Enzyme-based models for CUE and M, and modeled R_m exhibited significant differences among soil horizons, but no watershed or seasonal effects, with the exception of a watershed effect for M (Tables 4, 5).

Microbial EEA and DHA were significantly correlated with each other in both the bog and the fen, but with generally stronger correlations in the fen. The strongest correlations reflected the balance between C acquisition to fuel microbial metabolism, and nutrient acquisition to build proteins and nucleic acids, the threshold elemental ratio (TER; Frost et al. 2006). The weakest correlations were with POX, indicative of the recalcitrant nature of the phenolic compounds which POX degrades to yield more labile C products (Sinsabaugh and Follstad Shah 2011; Table 6A). The TER is also indicated by the strength of the correlations of C, N, and P acquiring EEA with microbial respiration (DHA; Table 6A).

Microbial EEA, DHA, vectors L or A, CUE, M, and R_m along the upland–peatland gradient were poorly correlated with outflow water chemistry from the bog, while these same microbial measures were more strongly correlated with outflow chemistry from the fen, reflecting the more isolated hydrology of the bog compared to the fen (Table 6B). The enzyme-based metrics were also correlated with the soil chemistry, soil stoichiometry, and LCI (Table 6C); however, they were not correlated with any of the atmospheric deposition, hydrology, or outflow chemistry variables listed in Table 1.

Microbial EEA stoichiometry was further evaluated using orthogonal regressions of ecoenzyme pairs normalized for POX (Fig. 1; Table 7). The slopes of the regressions lines indicate relative C-, N-, or P-limitations, with slopes >1 indicating enhancement of the EEA activity on the *y*-axis and slopes <1 indicating enhancement of the EEA activity on the *x*-axis. The deviation of the slope from a value of 1 is a measure of the relative degree of limitation. The relative C-, N-, and P-limitation are corroborated by the Ls and As of the vectors for (BG/[NAG + LAP])/

Table 3 Summary statistics of the Type III General Linear Model analysis of an unbalanced, nested sampling design for pore water and soil chemistry, soil stoichiometry, and the lignocellulose index (LCI) for the bog and the fen at the Marcell Experimental Forest

Variables	Effects	df	*F*	*P*
Outflow water chemistry				
Cl	Watershed	1	31.0	**<0.0001**
	Season	2	21.1	**<0.0001**
	Season × watershed	2	0.0323	1.69
DOC	Watershed	1	1113	**<0.0001**
	Season	2	63.6	**<0.0001**
	Season × watershed	2	57.1	**<0.0001**
TOC	Watershed	1	524	**<0.0001**
	Season	2	47.1	**<0.0001**
	Season × watershed	2	43.0	**<0.0001**
NO_3^-	Watershed	1	22.4	**<0.0001**
	Season	2	1.92	0.1479
	Season × watershed	2	0.65	0.5231
TP	Watershed	1	54.0	**<0.0001**
	Season	2	0.05	0.9472
	Season × watershed	2	0.17	0.8415
SO_4^{2-}	Watershed	1	11.8	**0.0006**
	Season	2	15.8	**<0.0001**
	Season × watershed	2	9.36	**0.0001**
Soil chemistry and stoichiometry				
pH	Watershed	1	36.2	**<0.0001**
	Season	2	8.67	**0.0002**
	Soil horizon	5	7.34	**<0.0001**
	Soil horizon × (watershed × season)	27	6.75	**<0.0001**
NH_4^+	Watershed	1	20.8	**<0.0001**
	Season	2	0.14	0.8662
	Soil horizon	5	17.5	**<0.0001**
	Soil horizon × (watershed × season)	27	2.17	**0.0007**
NO_3^-	Watershed	1	2.60	0.1077
	Season	2	0.66	0.5196
	Soil horizon	5	6.60	**<0.0001**
	Soil horizon × (watershed × season)	27	0.92	0.56807
TC	Watershed	1	49.3	**<0.0001**
	Season	2	0.40	0.6691
	Soil horizon	5	184	**<0.0001**
	Soil horizon × (watershed × season)	27	8.19	**<0.0001**
TN	Watershed	1	4.21	**0.0408**
	Season	2	11.3	**<0.0001**
	Soil horizon	5	9.57	**<0.0001**
	Soil horizon × (watershed × season)	27	3.30	**<0.0001**
TP	Watershed	1	5.25	**0.0013**
	Season	2	18.0	0.4493
	Soil horizon	5	0.95	**0.0006**
	Soil horizon × (watershed × season)	27	0.97	0.0851

Table 3 continued

Variables	Effects	df	*F*	*P*
Soil stoichiometry				
C/N	Watershed	1	8.45	**0.0038**
	Season	2	17.7	**<0.0001**
	Soil horizon	5	22.2	**<0.0001**
	Soil horizon × (watershed × season)	27	2.30	**0.0003**
C/P	Watershed	1	0	0.9481
	Season	2	3.67	**0.0262**
	Soil horizon	5	66.5	**<0.0001**
	Soil horizon × (watershed × season)	27	2.05	**0.0017**
N/P	Watershed	1	16.2	**<0.0001**
	Season	2	18.8	**<0.0001**
	Soil horizon	5	6.04	**<0.0001**
	Soil horizon × (watershed × season)	27	3.30	**<0.0001**
Lignocellulose index				
LCI	Watershed	1	6.48	**0.0112**
	Season	2	2.42	0.0896
	Soil horizon	5	23.4	**<0.0001**
	Soil horizon × (watershed × season)	27	1.00	0.4612

Samples were collected May–October 2010–2012. Significant watershed, season, soil horizon and interaction effects are indicate by bold *P* values

(BG/AP), where vector L increases with increasing enzyme allocation toward C acquisition and vector A increases with increasing allocation toward P acquisition relative to N acquisition (Table 4). The results of these analyses indicated significant differences between the bog and the fen watersheds, including much greater N- and P-limitation, relative to C acquisition in the bog compared to the fen (Fig. 1b, c, e, f; Table 7). Overall, both watersheds were relatively more P-limited than N- or C-limited.

The relationships between M, R_m, CUE, and DHA were investigated using orthogonal regressions (Figs. 1, 2; Table 8). Overall, CUE was a poor predictor of M in both the bog and the fen watersheds (Fig. 2a, b; Table 8), with the exception of the bog model (Table 8). R_m predicted from CUE yielded several significant models (Fig. 1c, d; Table 8), particularly for the bog. DHA was a significant, but weaker, predictor of M (Fig. 3a, b; Table 8). DHA was a better predictor of M in the uplands than in the bog or fen, and DHA was a significant predictor of R_m at the watershed, upland, and bog/fen model scales in both the bog and the fen watersheds (Fig. 3c, d; Table 8). This DHA–R_m relationship provided an independent validation of the enzyme-based model estimates for M and CUE.

Discussion

While estimates of microbial EEA from upland soils are widely reported (Sinsabaugh et al. 2008), few have been reported for peatlands (Freeman et al. 1996, 2004; Bragazza et al. 2006). Even fewer studies report EEA along depth profiles in either upland soils or peat layers. Our reported EEA values for the O-horizon of upland soils are similar to the global averages for BG, [NAG + LAP], AP, and POX from 40 ecosystems (Sinsabaugh et al. 2008). Likewise, our peat estimates for BG, [NAG + LAP], and AP are in the ranges of values reported for European peatlands, but our values for POX are considerably lower (Freeman et al. 1996, 2004; Bragazza et al. 2006). Bog and fen peat EEA are significantly greater than EEA for the upland soils of the watersheds, a result also reported for a comparison of EEA from upland soils with that from river

Table 4 Mean microbial respiration (DHA, nmol INT h^{-1} g^{-1} DW), ecoenzyme activities (nmol h^{-1} g^{-1} DW), enzyme allocation to C, N, and P acquisition (%), enzyme vector length (L, unitless) and angle (A, °), carbon use efficiency (CUE), predicted organic C decomposition rate (M, % day^{-1}), and estimated microbial respiration (R_m, mmol C m^{-3} day^{-1}) for the bog and the fen and their upland watersheds at the Marcell Experimental Forest

	Bog watershed						Fen watershed					
	Upland			Bog			Upland			Fen		
	O	A	B	SUR	ACR	CAT	O	A	B	SUR	ACR	CAT
Microbial respiration												
DHA	10,962	3,318	1,795	81,612	66,901	58,289	19,104	5,226	4,600	68,976	40,955	16,639
Ecoenzyme activity[a]												
BG	3,407	581	200	15,348	5,518	2,204	3,944	289	104	17,800	7,888	2,941
[NAG + LAP]	2,623	514	104	14,707	4,409	1,406	7,127	270	123	14,380	5,535	2,662
AP	4,639	948	270	38,168	8,083	4,110	12,307	647	311	58,375	21,249	6,826
POX	3,364	432	1,050	7,481	7,593	12,725	5,361	1,683	1,151	10,740	6,904	6,309
C, N, and P enzyme allocation												
C-acquisition	52	67	74	41	58	66	34	73	77	31	52	64
N-acquisition	17	10	7	16	15	9	26	11	8	14	10	8
P-acquisition	30	22	17	43	27	25	40	16	14	56	39	25
Vector L	1.42	1.47	1.50	1.38	1.43	1.46	1.24	1.34	1.29	1.36	1.37	1.35
Vector A	42.9	40.7	40.9	42.1	42.6	41.3	42.9	41.9	41.0	41.3	40.6	39.6
Enzyme-based decomposition model parameters												
CUE	0.16	0.22	0.28	0.07	0.04	0.03	0.13	0.13	0.25	0.10	0.08	0.10
M	0.014	0.005	0.003	0.020	0.009	0.004	0.020	0.002	0.003	0.026	0.014	0.006
R_m	5.72	0.74	0.02	24.3	13.9	8.63	9.92	0.04	0.01	26.7	12.1	8.17

Samples were collected May–October 2010–2012

[a] See text for full names of EEA

sediments and wetland soils (Sinsabaugh et al. 2009). These authors attributed the differences to relatively greater amounts of labile C in aquatic sediments and wetland soils compared to the more refractory C of the upland soils, a result of greater soil C age and microbial processing. This model of increasing C refraction with soil age is supported by our reported decreasing BG activities and increasing POX activities, presumably associated with increasing C refractivity and an increasing LCI with increasing soil or peat depth.

The increasing LCI with soil depth is also evident in the increasing allocation of enzymes toward C acquisition with increasing depth, mostly at the expense of P acquisition. Despite the large C pools, and the stoichiometric indication of N-limitation (soil C/N >16, peat C/N >24), a large proportion (31–77 %) of enzyme activity in these environments is for the acquisition of C. The proportion of enzyme activity allocated for N acquisition (7–26 %) is relatively constant across watersheds, uplands and bog/fen peat, and soil depth profiles, suggesting that N acquisition is less constraining on microbial growth or maintenance than either C or P acquisition. This finding is in agreement with results from a global analysis of soil EEA and C recalcitrance, which reported that microbial C demand increases faster than N or P demand as C recalcitrance increases (Sinsabaugh and Follstad Shah 2011). Moorhead et al. (2013) used the vectors L and A for the regression of (ln BG/ln [NAG + LAP]) against (ln BG/ln AP) to quantify the relative changes in C and P demand on decomposing leaf litter in sandy and loam soils. They reported increasing C demand (greater L) and decreasing P demand (decreasing A) with increasing L of time for decomposition (Moorhead et al. 2013). Our reported vectors L and A for soil depth profiles fit this same model, assuming greater depth equates to longer decomposition times.

Microbial C and nutrient demands are determined by the elemental stoichiometry of microbial biomass

Table 5 Summary statistics of the Type III General Linear Model analysis of an unbalanced, nested sampling design for microbial respiration (DHA, ln nmol h^{-1} g^{-1} DW), microbial ecoenzyme activities (ln nmol h^{-1} g^{-1} DW), enzyme allocation to C, N, and P acquisition (x-acq, %), enzyme vector length (L, unitless) and angle (A, °), modeled carbon use efficiency (CUE), organic C decomposition (M, % day^{-1}), and associated respiration (R_m, ln mmol C m^{-3} day^{-1}) for the bog and the fen at the Marcell Experimental Forest

Variable effects	df	*F*	*P*
Ecoenzyme activity			
DHA			
Watershed	1	0.19	0.6609
Season	2	1.38	0.2515
Soil horizon	5	61.0	**<0.0001**
Soil horizon × (watershed × season)	27	3.50	**<0.0001**
BG			
Watershed	1	7.97	**0.0050**
Season	2	1.06	0.3474
Soil horizon	95	185	**<0.0001**
Soil horizon × (watershed × season)	27	2.85	**<0.0001**
[NAG + LAP]			
Watershed	1	1.25	0.2651
Season	2	3.50	**0.0310**
Soil horizon	5	179	**<0.0001**
Soil horizon × (watershed × season)	27	1.85	**0.0067**
AP			
Watershed	1	5.94	**0.0152**
Season	2	4.11	**0.0170**
Soil horizon	5	201	**<0.0001**
Soil horizon × (watershed × season)	27	3.47	**<0.0001**
POX			
Watershed	1	0.46	0.4986
Season	2	0.14	0.8728
Soil horizon	5	9.04	**<0.0001**
Soil horizon × (watershed × season)	27	1.17	0.2546
C, N, and P enzyme allocation			
C-acq			
Watershed	1	12.2	**0.0005**
Season	2	1.57	0.2098
Soil horizon	5	48.4	**<0.0001**
Soil horizon × (watershed × season)	27	1.87	**0.0057**
N-acq			
Watershed	1	0.02	0.8758
Season	2	5.05	**0.0068**
Soil horizon	5	17.9	**<0.0001**
Soil horizon × (watershed × season)	27	1.03	0.4312
P-acq			
Watershed	1	15.3	**0.0001**
Season	2	6.51	**0.0016**
Soil horizon	5	37.5	**<0.0001**
Soil horizon × (watershed × season)	27	2.30	**0.0003**

Table 5 continued

Variable effects	df	*F*	*P*
L			
Watershed	1	60.4	**<0.0001**
Season	2	1.55	0.2130
Soil horizon	5	2.58	**0.0255**
Soil horizon × (watershed × season)	27	2.00	**0.0023**
A			
Watershed	1	2.58	0.1092
Season	2	24.6	**<0.0001**
Soil horizon	5	3.47	**0.0044**
Soil horizon × (watershed × season)	27	2.24	**0.0004**
Enzyme-based decomposition model parameters			
CUE			
Watershed	1	3.65	0.0567
Season	2	1.18	0.3083
Soil horizon	5	115	**<0.0001**
Soil horizon × (watershed × season)	27	1.54	**0.0428**
M			
Watershed	1	10.6	**0.0012**
Season	2	0.97	0.3813
Soil horizon	5	94.6	**<0.0001**
Soil horizon × (watershed × season)	27	2.12	**0.0010**
R_m			
Watershed	1	0.44	0.5088
Season	2	0.21	0.8077
Soil horizon	5	40.4	**<0.0001**
Soil horizon × (watershed × season)	27	1.74	**0.0128**

Samples were collected May–October 2010–2012. Significant watershed, season, soil horizon and interaction effects are indicate by *bold P* values

in relation to environmental C and nutrient availability (Schimel and Weintraub 2003; Cleveland and Liptzin 2007; Sinsabaugh et al. 2009). Ecological stoichiometric theory (EST) emphasizes the importance of the balance of biologically important elements for regulating an organism's response to, and regulation of, their environment (Sterner and Elser 2002). EEA represents the interface between microbial demands for, and environmental supplies of, C and nutrients, which effectively link the EST with the concept of TER (Frost et al. 2006). EST proposes that biomass production and nutrient retention are governed by elemental ratios and the stoichiometric invariance of biomass; TER is the critical C/N or C/P ratio at which metabolism switches from energy flow (C acquisition) to nutrient limitation (N or P acquisition). There are several models that link ecosystem–level interactions of organic matter, nutrients, and the role of microbial processes in the mediation of energy flow and nutrient cycling (Sinsabaugh and Moorhead 1994; Schimel and Weintraub 2003; Moorhead et al. 2012). These models highlight the interplay between microbial C acquisition to meet metabolic demands and the stoichiometric N and P requirements to support that level of metabolism. The interactions of C quantity and quality, nutrient supply, and EEA can be summarized as an inverse relationship between C recalcitrance and nutrient availability, resulting in decreasing microbial growth rate and increasing TER for N and P (Sinsabaugh and Follstad Shah 2011).

Table 6 Spearman rank correlation coefficients for the combined bog or fen upland–peatland gradients with (A) among microbial ecoenzyme activities (BG, [NAG + LAP], AP, POX) and respiration (DHA), (B) between microbial EEA, DHA, enzyme vector length (L) and angle (A), enzyme-based estimates of microbial carbon use efficiency (CUE), decomposition rate (M) and respiration (R_m), and outflow water chemistry, and (C) between microbial EEA, DHA, L, A, CUE, M, and R_m and soil C, N, and P chemistry and stoichiometry for the Marcell bog and fen (italics) watersheds

(A)

Sites	[NAG + LAP]	AP	POX	DHA
Microbial EEA and respiration				
BG				
Bog	0.85	0.77	0.17	0.38
Fen	*0.91*	*0.90*	*0.56*	*0.56*
[NAG + LAP]				
Bog		0.82	–	0.43
Fen		*0.90*	*0.55*	*0.81*
AP				
Bog			0.19	0.38
Fen			*0.52*	*0.78*
POX				
Bog				0.28
Fen				*0.51*

(B)

Sites	BG	[NAG + LAP]	AP	POX	DHA	L	A	CUE	M	R_m
Outflow water chemistry										
Cl^-										
Bog	–	–	−0.18	–	–	–	–	–	–	−0.35
Fen	–	–	–	–	–	–	–	–	–	–
DOC										
Bog	–	–	–	–	0.22	–	0.22	−0.26	–	–
Fen	*−0.16*	–	–	–	*−0.19*	–	–	–	–	–
TOC										
Bog	–	–	–	–	–	–	0.18	–	–	–
Fen	*−0.24*	*−0.20*	*−0.20*	–	*−0.24*	*−0.19*	–	–	–	*−0.16*
NO_3^-										
Bog	–	–	–	–	–	–	–	–	–	–
Fen	*−0.17*	–	*−0.15*	*−0.21*	*−0.22*	*−0.18*	–	*0.20*	–	–
TP										
Bog	–	0.20	–	–	–	−0.18	–	–	–	–
Fen	*−0.23*	*−0.22*	*−0.24*	*−0.19*	–	–	–	–	*−0.19*	*−0.17*
SO_4^{2-}										
Bog	–	–	–	–	−0.15	–	–	0.24	0.15	–
Fen	*0.17*	–	–	*0.19*	*0.28*	*0.19*	–	*−0.29*	–	–

(C)

Sites	BG	[NAG + LAP]	AP	POX	DHA	L	A	CUE	M	R_m
Soil chemistry and stoichiometry										
pH										
Bog	−0.17	−0.25	–	–	−0.56	–	−0.31	0.44	–	–
Fen	*−0.27*	*−0.30*	*−0.36*	–	–	–	–	–	*−0.37*	*−0.30*
NH_4^+										

Table 6 continued

(C)

Sites	BG	[NAG + LAP]	AP	POX	DHA	L	A	CUE	M	R_m
Bog	–	–	0.17	0.21	0.27	–	–	−0.60	–	0.37
Fen	*0.69*	*0.62*	*0.59*	*0.51*	*0.66*	*0.33*	*0.23*	*−0.46*	*0.54*	*0.70*
NO_3^-										
Bog	0.27	0.32	0.26	–	0.21	–	–	–	–	0.18
Fen	*0.46*	*0.51*	*0.50*	*0.20*	*0.48*	*–*	*0.15*	*–*	*0.45*	*0.49*
TC										
Bog	0.33	0.27	0.25	0.19	0.50	–	–	−0.62	–	0.47
Fen	*0.87*	*0.84*	*0.84*	*0.52*	*0.80*	*0.22*	*0.20*	*−0.38*	*0.75*	*0.89*
TN										
Bog	0.24	–	–	0.22	0.26	0.17	–	−0.61	–	0.47
Fen	*0.69*	*0.71*	*0.60*	*0.47*	*0.61*	*0.24*	*0.36*	*−0.16*	*0.70*	*0.82*
TP										
Bog	0.29	0.24	0.34	0.20	–	–	–	–	0.40	0.52
Fen	*0.53*	*0.49*	*0.48*	*0.36*	*0.50*	*0.16*	*0.22*	*–*	*0.63*	*0.55*
C/N										
Bog	0.45	0.58	0.53	0.21	0.64	−0.36	–	−0.33	0.21	0.36
Fen	*0.21*	*0.19*	*0.25*	*–*	*0.23*	*–*	*–*	*−0.39*	*–*	*–*
C/P										
Bog	0.29	0.32	0.23	0.18	0.51	–	–	−0.67	–	0.21
Fen	*0.75*	*0.72*	*0.73*	*0.50*	*0.72*	*0.18*	*0.17*	*−0.51*	*0.58*	*0.75*
N/P										
Bog	0.19	–	–	0.17	0.30	–	–	−0.69	−0.20	0.23
Fen	*0.50*	*0.54*	*0.43*	*0.37*	*0.43*	*–*	*0.29*	*−0.25*	*0.47*	*0.63*
LCI										
Bog	−0.66	−0.59	−0.48	0.54	−0.16	–	−0.22	–	−0.44	−0.43
Fen	*−0.78*	*−0.71*	*−0.70*	*–*	*−0.64*	*−0.32*	*−0.15*	*–*	*−0.62*	*−0.66*

Variable abbreviations and units are reported in Tables 2 and 4. Reported correlation coefficients are significant ($P < 0.05$ and $r > 0.15$). Non-significant correlations are indicated by dashes (–)

Our estimates of soil organic C decomposition based on EEA are within the ranges of decomposition rates (1–68 % mass loss $year^{-1}$) reported for other peatland studies (Farrish and Grigal 1988; Rochefort et al. 1990; Bragazza et al. 2012). In a previous decomposition study conducted in the same bog and fen as our current study, Farrish and Grigal (1988) measured mass losses from cellulose strips and bog peat buried for 1 year at depths corresponding to our surface, acrotelm, and catotelm layers. Peat decomposition rates in the bog were 5 % mass loss $year^{-1}$ (corresponds to M = 0.004 compared to our estimate of M = 0.020) for surface peat, 3 % loss $year^{-1}$ (M = 0.003 vs. our M = 0.003) for the acrotelm, and 1.5 % mass loss $year^{-1}$ (M = 0.0003 vs. our M = 0.004) for the catotelm. Cellulose decay along the peat depth gradient ranged from 21 % $year^{-1}$ (M = 0.008) to 5.5 % $year^{-1}$ (M = 0.004) in the bog compared to 65 % $year^{-1}$ (M = 0.011) to 26 % $year^{-1}$ (M = 0.008) in the fen (Farrish and Grigal 1988). Given similar depths for the catotelm layer between the bog and fen, these authors attributed the differences in decay rates to differences in pH. We also observed faster decomposition rates in the fen compared to the bog that may be related to higher pH in the fen.

Another explanation for lower decomposition rates is the presence of phenolic compounds that might inhibit hydrolytic enzyme activities. Freeman et al. (2004) reported that the addition of phenol oxidase (POX), an enzyme that degrades phenolic compounds, promoted significant increases in BG, NAG, and AP,

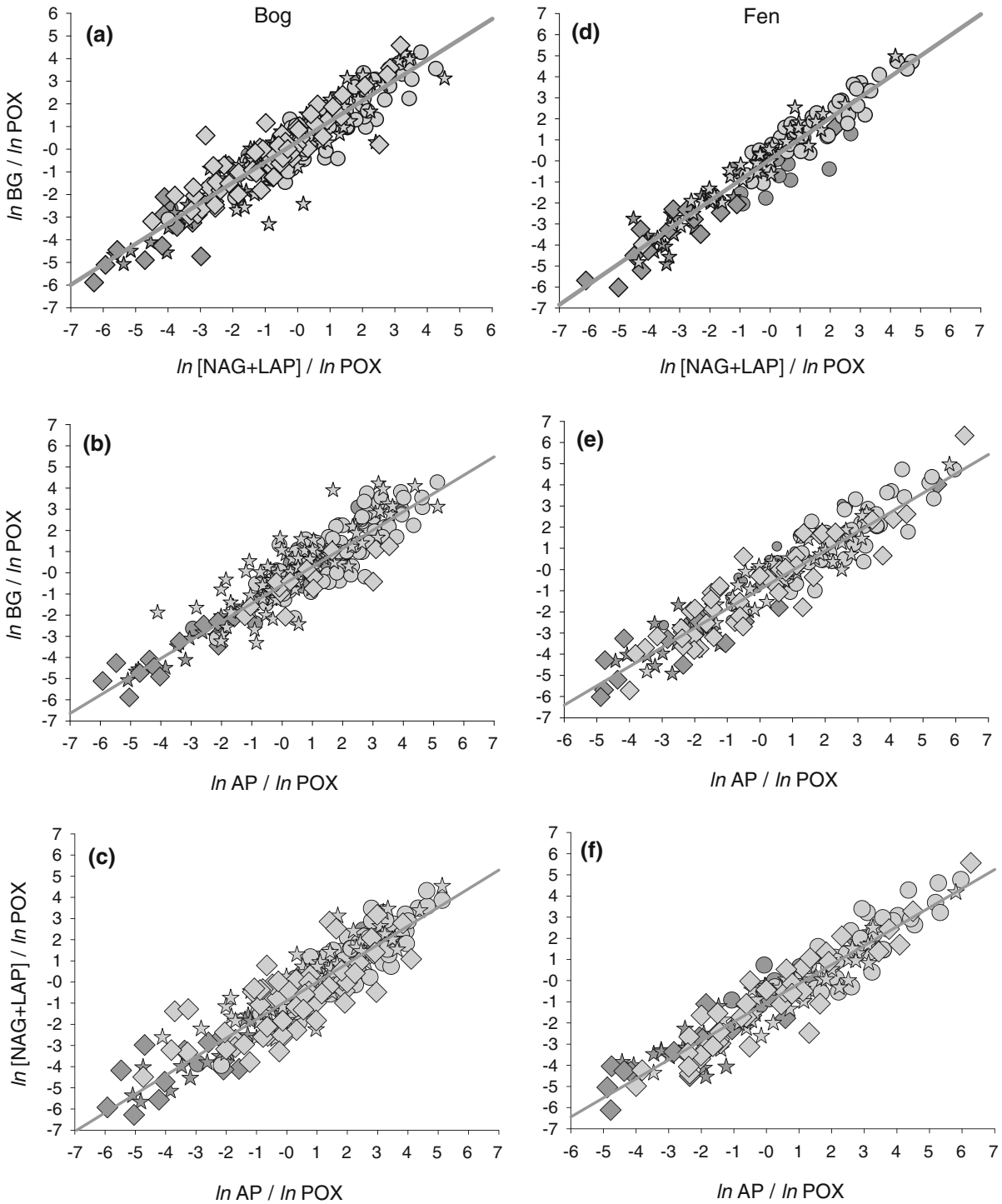

Fig. 1 POX-normalized ecoenzyme stoichiometry of C acquisition relative to N acquisition in the **a** bog upland and bog and **d** fen upland and fen, C acquisition relative to P acquisition in the **b** bog upland and bog and **e** fen upland and fen, and N acquisition relative to P acquisition in the **c** bog upland and bog and **f** fen upland and fen. *Dark gray dots*, *stars* and *diamonds* represent the O, A, and B horizons of upland soils, respectively, *light gray dots*, *stars* and *diamonds* represent the surface, acrotelm, and catotelm horizons, respectively, of the bog or fen peat. The *dark gray lines* represent the regression model for the entire watershed. Model results are presented in Table 7

Table 7 Results of orthogonal regression of stoichiometric relationships of POX-normalized C, N, and P acquiring EEA for the bog and fen watersheds at the Marcell Experimental Forest

WS	Location	Model df	Dependent Variables	Independent Variables	Slope (SE)	Intercept (SE)	r^2	P
Bog	Watershed	291	ln BG/ln POX	ln [NAG + LAP]/ ln POX	0.903 (0.020)	0.143 (0.020)	0.88	<0.0001
	Upland	57			0.892 (0.057)	0.159 (0.050)	0.81	<0.0001
	Bog	233			0.908 (0.022)	0.136 (0.023)	0.88	<0.0001
	Watershed	319	ln BG/ln POX	ln AP/ln POX	0.825 (0.022)	0.132 (0.025)	0.82	<0.0001
	Upland	63			0.899 (0.044)	0.056 (0.043)	0.87	<0.0001
	Bog	255			0.812 (0.026)	0.148 (0.030)	0.80	<0.0001
	Watershed	290	ln [NAG + LAP]/ ln POX	ln AP/ln POX	0.866 (0.023)	0.038 (0.026)	0.83	<0.0001
	Upland	57			0.850 (0.065)	0.035 (0.064)	0.75	<0.0001
	Bog	232			0.859 (0.025)	0.050 (0.030)	0.83	<0.0001
Fen	Watershed	173	ln BG/ln POX	ln [NAG + LAP]/ ln POX	0.953 (0.028)	0.052 (0.027)	0.87	<0.0001
	Upland	47			0.827 (0.071)	0.105 (0.062)	0.75	<0.0001
	Fen	125			0.952 (0.026)	0.074 (0.026)	0.92	<0.0001
	Watershed	182	ln BG/ln POX	ln AP/ln POX	0.837 (0.029)	0.046 (0.032)	0.82	<0.0001
	Upland	47			0.724 (0.071)	0.122 (0.070)	0.69	<0.0001
	Fen	136			0.888 (0.032)	0.05 (0.299)	0.85	<0.0001
	Watershed	172	ln [NAG + LAP]/ ln POX	ln AP/ln POX	0.840 (0.028)	0.030 (0.031)	0.84	<0.0001
	Upland	47			0.787 (0.067)	0.105 (0.066)	0.75	<0.0001
	Fen	124			0.880 (0.032)	−0.024 (0.032)	0.86	<0.0001

The watershed model includes both the upland and bog/fen data, the upland model includes only upland data, the bog/fen model includes only the bog/fen data. Significant watershed models are plotted in Fig. 2

and proposed that peatland decomposition was regulated by the relative abundance of POX in the environment. We did not observe significant differences in POX activity between the bog and the fen, but we did see higher AP activity in the fen compared to the bog. This higher rate of P-acquisition is indicative of increased microbial growth (Sinsabaugh et al. 2009), which might explain some of the modeled differences in decomposition rates.

We found good correspondence between our measured microbial respiration (DHA) and our modeled estimates of respiration (R_m) based on the available C pool and its BD, and the enzyme-based decomposition model. Our estimated R_m is similar to respiration rates measured in other peatland studies (6–25 mmol C m^{-3} day^{-1}; Farrish and Grigal 1988; Vile et al. 2003; Dorrepaal et al. 2009; Fenner et al. 2011). As was the case with our R_m, respiration declined with increasing depth (Farrish and Grigal 1988; Vile et al. 2003). The results from these other studies and our study indicate that significant decomposition and respiratory C losses occur in the anaerobic sub-surface layers in addition to the surface layers of peatlands, and these sub-surface layers should not be ignored when accounting for C sequestration in peatland ecosystems.

Sinsabaugh et al. (2009) used EEA stoichiometry to demonstrate the interaction of EST, TER, and the metabolic theory of ecology (MTE; Allen and Gillooly 2009). MTE states that metabolism is the means by which organisms interact with their environments and the collective organisms' metabolism scales to the ecosystem–level through energetic invariance, as evidenced by the positive correlation of our DHA measures and our estimated R_m for upland soils and peat (Fig. 2c, d). Sinsabaugh et al. (2009) used these models to predict microbial biomass C, N, and P from EEA stoichiometry. As such, the relative activities of the functional classes of ecoenzymes are both a measure of nutrient availability and of ecosystem

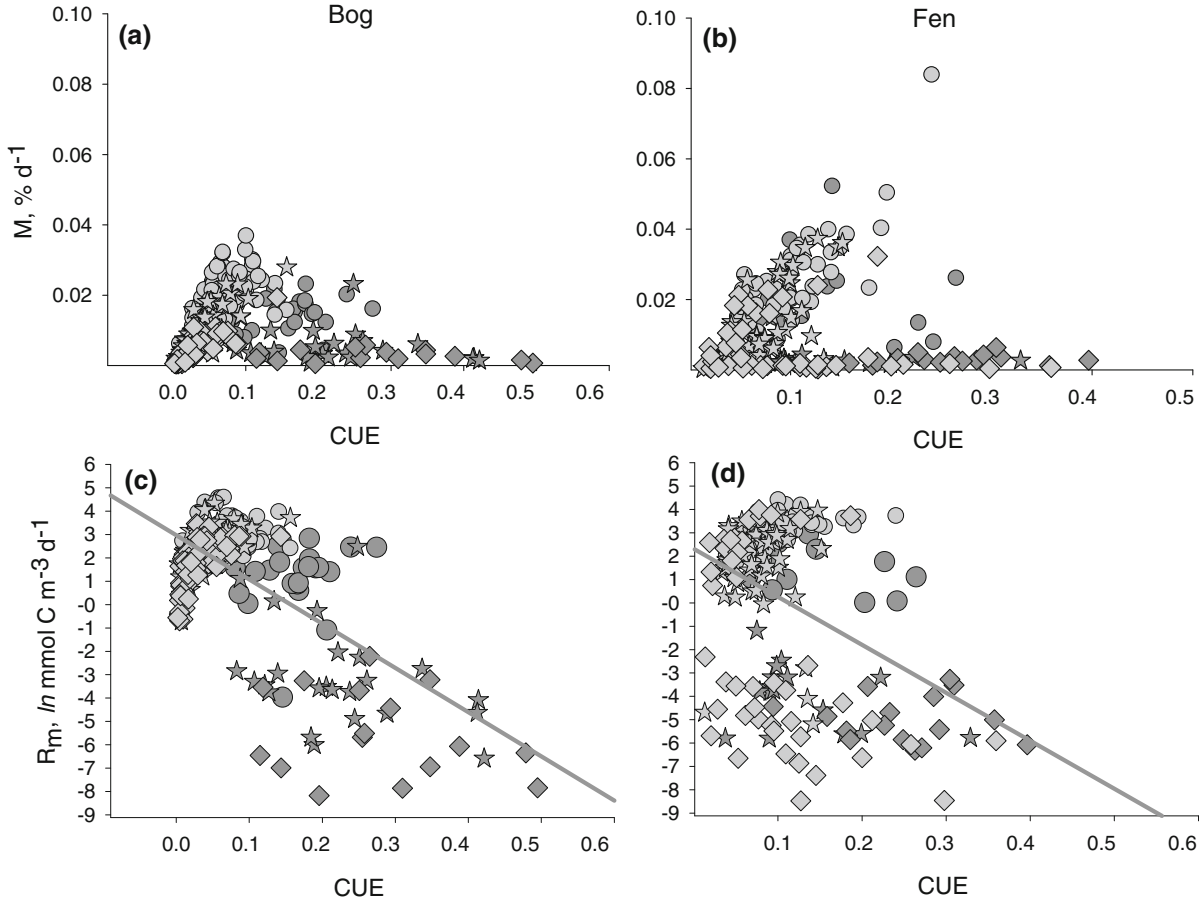

Fig. 2 Modeled decomposition rate (M, % day^{-1}; **a**, **b**) and respiration (R_m, mmol C m^{-3} day^{-1}; **c**, **d**) versus microbial carbon use efficiency (CUE) for upland and peat samples from the bog and fen watersheds. *Dark gray dots*, stars and *diamonds* represent the O, A, and B horizons of upland soils, respectively, *light gray dots*, *stars* and *diamonds* represent the surface, acrotelm, and catotelm horizons, respectively, of the bog or fen peat. The *dark gray lines* represent the regression model for the entire watershed. Model results are presented in Table 8

metabolism that may be used to assess large-scale phenomena such as regional impacts of anthropogenic disturbances.

The vast stores of C in peatlands are largely the result of 10–12 millennia of primary production exceeding respiration (Bridgham et al. 2008; Limpens et al. 2008). While peatlands continue to accumulate C at globally averaged rates of 200–600 kg ha^{-1} $year^{-1}$ (Limpens et al. 2008), their greatest potential impact on the global C budget and climate is as a source of C to the atmosphere upon decomposition of this accumulated peat (Gorham 1991; Bridgham et al. 2008; Limpens et al. 2008). Limpens et al. (2008) reviewed four models of C balances for peatlands and concluded that the majority of C taken up by peatland vegetation was released via respiration and decomposition, processes which are positively correlated with temperature and negatively correlated with water table levels (Bridgham et al. 2008; Limpens et al. 2008; Dorrepall et al. 2009). Most climate change models project temperature increases at northern latitudes and precipitation decreases in mid-continental areas (Solomon et al. 2007). Our measured and R_m rates indicate significant C losses from both bog and fen peatlands, even from the anaerobic catotelm layer, which may increase with rising temperatures and lowering water tables, but which may also be

Table 8 Results of orthogonal regression of stoichiometric relationships between CUE, M, and DHA for the bog and fen watersheds at the Marcell Experimental Forest

WS	Location	Model df	Dependent Variables	Independent Variables	Slope (SE)	Intercept (SE)	r^2	P
Bog	Watershed	318	M	CUE	0.624 (0.573)	0.073 (0.008)	<0.01	0.2772
	Upland	63			−4.946 (1.841)	0.252 (0.019)	0.10	0.0093
	Bog	254			2.910 (0.146)	0.013 (0.002)	0.61	<0.0001
	Watershed	318	ln R_m	CUE	−0.034 (0.004)	0.152 (0.009)	0.20	<0.0001
	Upland	63			−0.037 (0.012)	0.239 (0.015)	0.12	0.0042
	Bog	254			0.022 (0.002)	−0.009 (0.005)	0.34	<0.0001
	Watershed	317	M	ln DHA	0.0007 (0.0003)	0.003 (0.003)	0.02	0.0221
	Upland	63			0.005 (0.0006)	−0.029 (0.005)	0.44	<0.0001
	Bog	253			−0.0005 (0.0005)	0.017 (0.005)	<0.01	0.2543
	Watershed	317	ln R_m	ln DHA	0.328 (0.037)	−1.219 (0.381)	0.20	<0.0001
	Upland	63			0.586 (0.105)	−4.405 (0.858)	0.34	<0.0001
	Bog	253			−0.097 (0.046)	3.536 (0.499)	0.02	0.0361
Fen	Watershed	183	M	CUE	−0.053 (0.424)	0.113 (0.008)	<0.01	0.9011
	Upland	47			−1.801 (1.236)	0.186 (0.017)	0.04	0.1520
	Fen	135			1.136 (0.341)	0.074 (0.007)	0.08	0.0011
	Watershed	183	ln R_m	CUE	−0.015 (0.004)	0.139 (0.008)	0.09	<0.0001
	Upland	47			−0.029 (0.012)	0.191 (0.015)	0.12	0.0165
	Fen	135			−0.001 (0.003)	0.094 (0.009)	<0.01	0.7510
	Watershed	182	M	ln DHA	0.006 (0.0006)	−0.046 (0.006)	0.39	<0.0001
	Upland	47			0.008 (0.002)	−0.066 (0.013)	0.40	<0.0001
	Fen	134			0.006 (0.0007)	−0.043 (0.007)	0.35	<0.0001
	Watershed	182	ln R_m	ln DHA	0.830 (0.053)	−6.273 (0.513)	0.58	<0.0001
	Upland	47			0.821 (0.162)	−6.515 (1.428)	0.36	<0.0001
	Fen	134			0.756 (0.060)	−5.429 (0.606)	0.54	<0.0001

The watershed model includes both the upland and bog/fen data, the upland model includes only upland data, the bog/fen model includes only the bog/fen data. Significant watershed models are plotted in Figs. 2 and 3

constrained by C, N, or P availability. Microbial respiration, EEA, and decomposition metrics were not correlated with atmospheric deposition of C, N, or P, but were strongly correlated with soil pools of these elements, suggesting a more localized control of elemental acquisition to support microbial biomass maintenance and accumulation. The implications of a localized control suggest that the interactions of site-scale resource availability and microbial EEA may not scale linearly to regional responses to changing temperature and precipitation regimes, with some authors suggesting that temperature and drying may work in opposition with each other, which would result in no discernible change in C storage in peatlands in mid-continental landscapes (Bridgham et al. 2008; Limpens et al. 2008; Seifert-Monson 2013).

The results of our enzyme-based approach for modeling C, N, and P acquisition, respiration, and organic matter decomposition, all rooted in stoichiometric theory, suggests that this is a valid approach for modeling resource limitations on microbial metabolism and biogeochemical cycling in peatlands. Enzymatic stoichiometry models are also a cost-effective tool for monitoring ecosystem responses to resource availability and the environmental drivers of microbial metabolism, including those related to global climate changes.

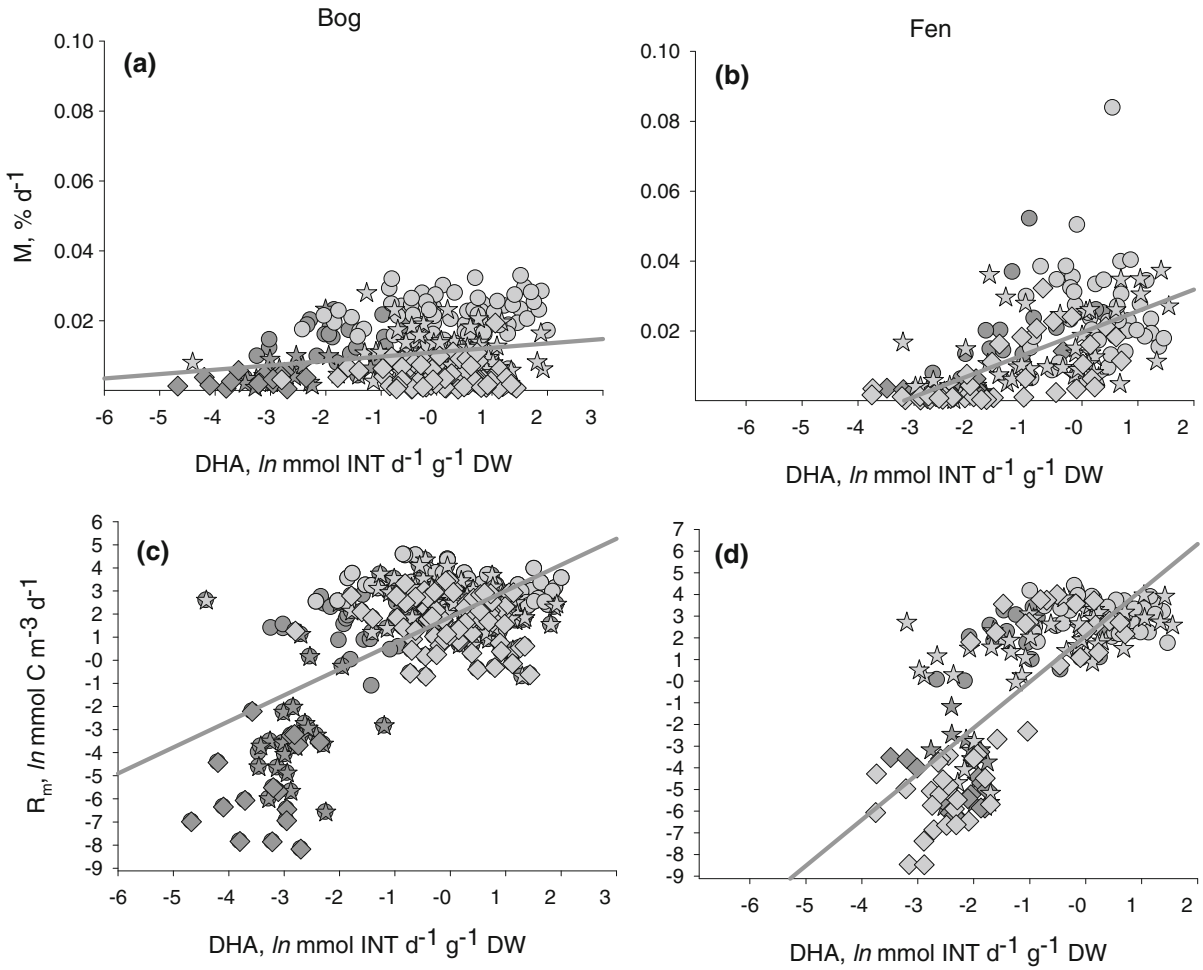

Fig. 3 Modeled decomposition rate (M, % day^{-1}; **a**, **b**) and respiration (R_m, mmol C m^{-3} day^{-1}; **c**, **d**) versus measured microbial respiration (DHA, mmol INT day^{-1} g^{-1} DW) for upland and peat samples from the bog and fen watersheds. *Dark gray dots*, *stars* and *diamonds* represent the O, A, and B horizons of upland soils, respectively, *light gray dots*, *stars* and *diamonds* represent the surface, acrotelm, and catotelm horizons, respectively, of the bog or fen peat. The *dark gray lines* represent the regression model for the entire watershed. Model results are presented in Table 8

Acknowledgments The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency or the US Forest Service. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References

Alexander RB, Bohlke JK, Boyer EW, David MB, Harvey JW, Mulholland PJ, Seitzinger SP, Tobias CR, Tonitto C, Wollheim WM (2009) Dynamic modeling of nitrogen losses in river networks unravels the coupled effects of hydrological and biogeochemical processes. Biogeochemistry 93:91–116

Allen AP, Gillooly JF (2009) Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. Ecol Lett 12:369–384

American Public Health Association, APHA (1998) In: Clesceri LS, Greenberg AE, Eaton AD (eds) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC

Arshad MA, Lowery B, Grossman B (1996) Physical tests for monitoring soil quality. In: Doran JW, Jones AJ (eds) Methods for assessing soil quality. Soil Science Society of America, Madison, pp 123–141

Bragazza L, Freeman C, Jones T, Rydin H, Limpens J, Fenner N, Ellis T, Gerdol R, Hajek M, Hajek T, Iacumin P, Kutnar L, Tahvanainen T, Toberman H (2006) Atmospheric nitrogen deposition promotes carbon loss from peat bogs. Proc Natl Acad Sci USA 103:19386–19389

Bragazza L, Buttler A, Habermacher J, Brancaleoni L, Gerdol R, Fritze H, Hanajik P, Laiho R, Johnson D (2012) High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. Glob Change Biol 18:1163–1172

Bridgham SD, Pastor J, Janssens JA, Chapin C, Malterer TJ (1996) Multiple limiting gradients in peatlands: a call for a new paradigm. Wetlands 16:45–65

Bridgham SD, Updegraff K, Pastor J (1998) Carbon, nitrogen, and phosphorus mineralization in northern wetlands. Ecology 79:1545–1561

Bridgham SD, Megonigal JP, Keller JK, Bliss NB, Trettin C (2006) The carbon balance of North American wetlands. Wetlands 26:889–916

Bridgham SD, Pastor J, Dewey B, Weltzin JF, Updegraff K (2008) Rapid carbon response of peatlands to climate change. Ecology 89:3041–3048

Broberg A (1985) A modified method for studies of electron transport system activity in freshwater sediments. Hydrobiologia 120:181–187

Cleveland CC, Liptzin D (2007) C/N/P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85:235–252

Dorrepaal E, Toet S, van Lotestijn RSP, Swart E, van de Weg MJ, Callaghan TV, Aerts R (2009) Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. Nature 460:616–619

Farrish KW, Grigal DF (1988) Decomposition in an ombrotrophic bog and a minerotrophic fen in Minnesota. Soil Sci 145:353–358

Fenner N, Williams R, Toberman H, Hughes S, Reynolds B, Freeman C (2011) Decomposition 'hotspots' in a rewetted peatland: implications for water quality and carbon cycling. Hydrobiologia 674:51–66

Foreman CM, Franchini P, Sinsabaugh RL (1998) The trophic dynamics of riverine bacterioplankton: relationships among substrate availability, ectoenzyme kinetics and growth. Limnol Oceanogr 43:1344–1352

Freeman C, Liska NJ, Lock MA, Reynolds B, Hudson J (1996) Microbial activity and enzymic decomposition processes following peatland water table drawdown. Plant Soil 180:121–127

Freeman C, Ostle N, Fenner N, Kang H (2004) A regulatory role for phenol oxidase during decomposition in peatlands. Soil Biol Biochem 36:1663–1667

Frost PC, Benstead JP, Cross WF, Hillebrand H, Larson JH, Xenopoulos MA, Yoshida T (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecol Lett 9:774–779

German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397

Gorham E (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecol Appl 1:182–195

Gorham E, Eisenreich SJ, Ford J, Santelmann MV (1985) The chemistry of bog waters. In: Stumm W (ed) Chemical processes in lakes. Wiley, New York, pp 339–363

Hayden MJ, Ross DS (2005) Denitrification as a nitrogen removal mechanism in a Vermont peatland. J Environ Qual 34:2052–2061

Hedin LO, Armesto JJ, Johnson AH (1995) Patterns of nutrient loss from unpolluted, old-growth temperate forests: evaluation of biogeochemical theory. Ecology 76:493–509

Hill BH, Elonen CM, Jicha TM, Cotter AM, Trebitz AS, Danz NP (2006) Sediment microbial enzyme activity as an indicator of nutrient limitation in Great Lakes coastal wetlands. Freshw Biol 51:1670–1683

Kayranli B, Scholz M, Mustafa A, Hedmark A (2010) Carbon storage and fluxes within freshwater wetlands: a critical review. Wetlands 30:111–124

Keeney DR, Nelson DW (1986) Nitrogen: inorganic forms. In: Page AL (ed) Methods of soil analysis. Part 2. Chemical and microbiological properties. Agronomy monograph 9, 2nd edn. American Society of Agronomy and Soil Science Society of American, Madison, pp 643–649

Keiblinger KM, Hall EK, Wanek W, Szukics U, Hammerle I, Ellerdorfer G, Bock S, Straus J, Sterflinger K, Richter A, Zechmeister-Boltenstern S (2010) The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. FEMS Microbiol Ecol 73:430–440

Keller JK, Bridgham SD (2007) Pathways of anaerobic carbon cycling across an ombrotrophic–minerotrophic peatland gradient. Limnol Oceanogr 52:96–107

Keller JK, Bauers AK, Bridgham SD, Kellogg LE, Iversen CM (2006) Nutrient control of microbial carbon cycling along an ombrotrophic–minerotrophic peatland gradient. J Geophys Res 111:G03006, 1–14

Kellogg LE, Bridgham SD (2003) Phosphorus retention and movement across an ombrotrophic–minerotrophic peatland gradient. Biogeochemistry 63:299–315

Lachat Instruments (2009) Methods list for automated ion analyzers: flow injection, ion chromatography. Lachat Instruments, Loveland

Limpens J, Berendse F, Blodau C, Canadell JG, Freeman C, Holden J, Roulet N, Rydin H, Schaepman-Strub G (2008) Peatlands and the carbon cycle: from local processes to global implications—a synthesis. Biogeosciences 5:1475–1491

Manzoni S, Taylor P, Richter A, Porporato A, Agren GI (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. N Phytol 196:79–91

Mitchell PJ, Branfireum BA, Kolka RK (2008) Spatial characteristics of net methylmercury production hot spots in peatlands. Environ Sci Technol 42:1010–1016

Moorhead DL, Lashermes G, Sinsabaugh RL (2012) A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. Soil Biol Biochem 53:133–141

Moorhead DL, Rinkes ZL, Sinsabaugh RL, Weintraub MN (2013) Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. Front Microbiol 4:223.1–223.12

Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:205–221

Rochefort L, Vitt DH, Bayley SE (1990) Growth, production, and decomposition dynamics of *Sphagnum* under natural and experimentally acidified conditions. Ecology 71: 1986–2000

Schimel JP, Weintraub MN (2003) The implications or exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563

Sebestyen SD, Verry ES (2011) Effects of watershed experiments on water chemistry at the Marcell Experimental Forest. In: Kolka RK, Sebestyen SD, Verry ES, Brooks KN (eds) Peatland biogeochemistry and watershed hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, pp 433–458

Sebestyen SD, Dorrance C, Olson DM, Verry ES, Kolka RK, Elling AE, Kyllander R (2011) Long-term monitoring sites and trends at the Experimental Forest. In: Kolka RK, Sebestyen SD, Verry ES, Brooks KN (eds) Peatland biogeochemistry and watershed hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, pp 16–71

Seifert-Monson LR (2013) Effects of altered deposition chemistry on dissolved organic carbon, pore water nutrients, and microbial enzyme activity in a northern peatland. Master's Thesis, University of Minnesota, Minneapolis

Sinsabaugh RL, Follstad Shah JJ (2011) Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. Biogeochemistry 102:31–43

Sinsabaugh RL, Follstad Shah JJ (2012) Ecoenzymatic stoichiometry and ecological theory. Annu Rev Ecol Evol Syst 43:313–343

Sinsabaugh RL, Foreman CM (2001) Activity profiles of bacterioplankton in a eutrophic river. Freshw Biol 46: 1239–1249

Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biol Biochem 26:1305–1311

Sinsabaugh RL, Findlay S, Franchini P, Fisher D (1997) Enzymatic analysis of riverine bacterioplankton production. Limnol Oceanogr 42:29–38

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursove M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH (2008) Stoichiometry of soil enzyme activity at global scale. Ecol Lett 11:1252–1264

Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial nutrient acquisition in soil and sediment. Nature 462:795–798

Sinsabaugh RL, Follstad Shah JJ, Hill BH, Elonen CM (2012) Ecoenzymatic stoichiometry of stream sediments with comparison to terrestrial soils. Biogeochemistry 111:455–467

Sinsabaugh RL, Manzoni S, Moorhead DL, Richter A (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modeling. Ecol Lett 16:930–939

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) (2007) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton

Stoddard JL (1994) Long-term changes in watershed retention of nitrogen: its causes and aquatic consequences. In: Baker LA (ed) Environmental chemistry of lakes and reservoirs. Advances in chemistry series number 237. American Chemical Society, Washington, DC, pp 223–284

Trevors JT, Mayfield CI, Inniss WE (1982) Measurement of electron transport system (ETS) activity in soil. Microb Ecol 8:163–168

Urban NR, Eisenreich SJ, Bayley SE (1988) The relative importance of denitrification and nitrate assimilation in midcontinental bogs. Limnol Oceanogr 33:1611–1617

Urban NR, Verry ES, Eisenreich SJ, Grigal DF, Sebestyen SD (2011) Element cycling in upland/peatland watersheds. In: Kolka RK, Sebestyen SD, Verry ES, Brooks KN (eds) Peatland biogeochemistry and watershed hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, pp 213–241

Verry ES, Timmons DR (1977) Precipitation nutrients in the open and under two forests in Minnesota. Can J For Res 7:112–119

Verry ES, Timmons DR (1982) Waterbourne nutrient flow through an upland–peatland watershed in Minnesota. Ecology 63:1456–1467

Verry ES, Brooks KN, Nichols DS, Ferris DR, Sebestyen SD (2011a) Watershed hydrology. In: Kolka RK, Sebestyen SD, Verry ES, Brooks KN (eds) Peatland biogeochemistry and watershed hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, pp 193–212

Verry ES, Bay RR, Boelter DH (2011b) Establishing the Marcell Experimental Forest: threads in time. In: Kolka RK, Sebestyen SD, Verry ES, Brooks KN (eds) Peatland biogeochemistry and watershed hydrology at the Marcell Experimental Forest. CRC Press, Boca Raton, pp 1–13

Vile MA, Bridgham SD, Wieder RK (2003) Response of anaerobic carbon mineralization rates to sulfate amendments in a boreal peatland. Ecol Appl 13:720–734