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Microbial ecoenzyme stoichiometry, nutrient limitation, and organic matter decomposition in wetlands of the conterminous United States

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Abstract

Microbial respiration (R_m) and ecoenzyme activities (EEA) related to microbial carbon, nitrogen, and phosphorus acquisition were measured in 792 freshwater and estuarine wetlands (representing a cumulative area of 217,480 km²) across the continental United States as part of the US EPA's 2011 National Wetland Condition Assessment. EEA stoichiometry was used to construct models for and assess nutrient limitation, carbon use efficiency (CUE), and organic matter decomposition ($-k$). The wetlands were classified into ten groups based on aggregated ecoregion and wetland type. The wetlands were also assigned to least, intermediate, and most disturbed classes, based on the extent of human influences. Ecoenzyme activity related to C, N and P acquisition, R_m , CUE, and $-k$ differed among ecoregion-wetland types and, with the exception of C acquisition and $-k$, among disturbance classes. R_m and EEA were positively correlated with soil C, N and P content ($r = 0.15$ – 0.64) and stoichiometry ($r = 0.15$ – 0.48), and negatively correlated with an index of carbon quality ($r = -0.22$ to -0.39). EEA stoichiometry revealed that wetlands were more often

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P- than N-limited, and that P-limitation increases with increasing disturbance. Our enzyme-based approach for modeling C, N, and P acquisition, and organic matter decomposition, all rooted in stoichiometric theory, provides a mechanism for modeling resource limitations of microbial metabolism and biogeochemical cycling in wetlands. Given the ease of collecting and analyzing soil EEA and their response to wetland disturbance gradients, enzyme stoichiometry models are 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.

Keywords

Climate; Decomposition; Ecoenzymes; Respiration; Soil; Stoichiometry; Wetlands

Introduction

Wetlands are a significant component in the global carbon budget because of the quantity of carbon (C) stored in their soils. Global wetland C storage is estimated to be 350–535 Pg (Pg = 10^{15} g), with wetlands of the conterminous United States storing 11.5 Pg C (Mitra et al. 2005; Bridgman et al. 2006; Nahlik and Fennessy 2016). The amount of C sequestration in wetlands depends on nitrogen (N) and phosphorus (P) limitations on primary productivity and on rates of organic matter decomposition. Microbial coenzyme activities may reflect carbon dynamics resulting from decomposition processes and might be useful indicators of ecosystem services related to carbon and nutrient sequestration (Bedford et al. 1999; Hill et al. 2006; Sinsabaugh et al. 2009).

Microbial respiration (R_m) related to biomass maintenance and organic matter decomposition is the primary pathway for organic C mineralization in wetland soils. Microbial assemblages also produce coenzymes to facilitate organic matter decomposition and to acquire organically-bound carbon and nutrients (Sinsabaugh and Foreman 2001; Hill et al. 2006; Sinsabaugh et al. 2009). Sinsabaugh et al. (2009) suggested the term coenzyme to describe enzymes that were excreted or lysed from cells and located outside of the cell membrane.

This current study uses microbial coenzyme activity (EEA) stoichiometry, the relative abundances of C, N and P acquiring EEA, as indicators of nutrient limitations on microbial assemblages (Hill et al. 2006, 2014; Sinsabaugh et al. 2009). Microbial C acquisition is regulated by the size of the labile C pool in the environment, as constrained by the availability of N and P. The molar stoichiometry of the C/N/P pool in aquatic sediments approximates 186C/13N/1P, while that of microbial biomass should be near 60C/ 7N/1P (Cleveland and Liptzin 2007). Deviations from expected C/N/P indicate that microbial metabolism and growth may be limited by the element in short supply, and this limitation will be hierarchically transferred through organisms, populations, and ecosystems (Sinsabaugh et al. 2009).

The relative apportionment of microbial C acquisition toward growth versus metabolic maintenance (respiration) of existing biomass is termed C use efficiency (CUE; Keiblinger et al. 2010; Manzoni et al. 2012; Sinsabaugh et al. 2013). CUE, which ranges from 0 to 0.60,

is negatively correlated with temperature and with C, N and P availability (Manzoni et al. 2012; Sinsabaugh et al. 2013; Hill et al. 2014). Microbial EEA stoichiometry and CUE has also been used to infer organic matter decomposition in streams (Sinsabaugh and Moorhead 1994) and peat bogs (Hill et al. 2014), and decomposition rates were positively correlated with soil C, N and P concentrations and inversely correlated with soil depth (Hill et al. 2014).

The objectives of the NWCA survey were to assess the ecological condition of a statistical representation of the Nation's wetlands. The specific goals for the enzyme study were to investigate the role of microbial EEA in C, N and P acquisition to support microbial metabolism across the wide variety of wetlands in the continental US. We hypothesized that microbial EEA would reflect the relative availability of C, N and P in wetland sediments and that we could use the relative carbon (C) and nutrient acquiring EEA to estimate relative C, N, and P limitations on microbial metabolism. We then used enzyme decomposition models to estimate microbial carbon use efficiencies (CUE) and organic matter decomposition rates ($-k$) in wetlands at regional to national scales. Lastly, we explored microbial responses (R_m , EEA, CUE, $-k$) to different levels of anthropogenic disturbance in these wetlands.

Methods

Study sites

This study was a part of the US Environmental Protection Agency Office of Water's National Wetland Condition Assessment (NWCA), which was conducted in 2011. The NWCA sampled 1138 wetland sites in the conterminous United States. Of those, 967 were probability sites used to infer wetland condition across the US, the remaining 171 sites were hand-picked reference sites or state assessment sites that we used in the analysis to fill in the stressor gradients but not for making population estimates. Probability sites in the NWCA were chosen from a sample frame composed of wetland areas identified in the US Fish and Wildlife Service National Wetlands Inventory's Status and Trend (S&T) program (Dahl and Bergeson 2009; Dahl 2011). NWCA random wetland sample points were selected from the wetland designated polygons within the S&T plots using a General Random Tessellation Stratified survey design (Olsen et al. in press; Stevens and Olsen 2004). The design ensures that sampled wetlands have a known probability of inclusion and that their distribution is spatially balanced. The power of this survey design is that it ensures an unbiased areal estimate of the condition (with known probability and error rates) of the targeted wetland resources over a large geographic area despite the relatively small number of samples collected. The NWCA wetlands have rooted vegetation and, when present, open water less than 1 m deep. The NWCA design allows the assessment of ecological conditions of wetlands at multiple hierarchical spatial scales, and/or by wetland types (USEPA 2016).

Wetland sites were sampled in 2011 during an index period ranging from April to September depending on the growing season of the state in which the site was located (USEPA 2016). Sample collection focused on a 0.5-ha assessment area defined around each randomly selected sample point. The assessment area was generally circular with a 40 m radius, except for very small or narrow wetlands where the shape was adjusted to fit within the wetland boundary. Here we report results for 10 aggregated ecoregion-wetland types (Table 1a) and

by disturbance class (least, intermediate, most). Disturbance classes were defined based on screening 10 wetland disturbance metrics including, the extent of human influences and hydro-logic modification in the assessment area, alien plant cover, and soil heavy metals (see Herlihy et al. 2008 for details). Sampling constraints at some sites prevented the entire suite of data from being collected, and we report here the results from 792 NWCA wetland probability sites, representing a cumulative area of 217,480 km², for which we had complete soil chemistry, microbial respiration and ecoenzyme activity data (Fig. 1).

Soil collection and chemistry

A soil sample from each wetland was obtained by combining three 10-cm deep cores (1.24 cm diameter) from an area deemed by the field crews as representative of that wetland assessment area (USEPA 2011). We restricted our analyses to the upper 10 cm of soils, because we felt this stratum was likely to be the most microbiologically active, as has been previously reported (Hill et al. 2014). Soil samples were stored frozen (− 20 °C) until thawed for analyses, then dried and ground for total carbon (TC), total nitrogen (TN), and total phosphorus (TP) analyses. Soil TC and TN were analyzed by combustion using a Model 1112 EA Carla Erba elemental analyzer; soil TP was determined by first digesting the sample in reagent grade concentrated nitric acid (HNO₃) using a CEM Corporation microwave, then neutralizing with sodium hydroxide (NaOH) and analyzing by the molybdate-ascorbic acid method (Method 10–107–04–1: Lachat Instruments 2009). Soil moisture content was determined by gravimetric methods using a drying oven at 60 °C to a constant weight. The percent solids were used to calculate available nutrient content on a dry weight basis. Soil C/N/P were calculated based on molar C, N and P values.

Microbial respiration and ecoenzyme activity

We analyzed soil samples for microbial respiration (as dehydrogenase activity) and ecoenzyme activity related to C, N, and P acquisition (Table 1b). Duplicate dehydrogenase aliquots (1 g wet weight) were mixed with 2.5 mL of sodium bicarbonate (NaHCO₃, pH 8.1) buffer and 1 mL of 0.75% 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) standard, sealed, agitated, and incubated (dark, 27 °C) for 1.5 h. Aliquots were then centrifuged (2000 × *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 dry weight to calculate dehydrogenase activity (nmol INT h^{−1} g^{−1} DW). On a molar basis, 2 mol of INT are equivalent to 1 mol of CO₂ respired (Broberg 1985), so DHA was multiplied by the dividend of the molecular weight of CO₂ (44) divided by the molecular weight of INT (471) to yield an estimate of microbial respiration (R_m, nmol C h^{−1} g^{−1} DW):

$$R_m = (\text{INT}/2) \times (44/471). \quad (1)$$

Carbon acquisition was measured as the activity of five glycosidases: α-D-galactosidase (AGAL, Enzyme Commission number [EC] 3.2.1.22), β-D-galactosidase (BGAL, EC 3.2.1.23), β-D-glucosidase (BG, EC 3.2.1.21), cellobiohydrolase (CBH, EC 3.2.1.91), and β-D-xylosidase (BX, EC 3.2.1.8). Nitrogen acquisition was measured as the activity of β-N-

acetylglucosaminidase (NAG; EC 3.2.1.50) and two aminopeptidases, L-alanine aminopeptidase (AAP, EC 3.4.11.2) and L-leucine aminopeptidase (LAP, EC 3.4.11.1). Phosphorus acquisition was measured as acid phosphatase (AP, EC 3.1.3.2) activity. The glycosidase, glucosaminidase, aminopeptidase, and phosphatase assays used substrates linked to methyl-umbelliferyl or methyl-coumarin residues (Sigma-Aldrich Corporation, St. Louis, MO, USA). Polyphenol oxidase (POX, EC 1.14.18.1) was analyzed to assess the relative importance of recalcitrant C processing by wetland microbial assemblages. The polyphenol oxidase assay used l-3,4-dihydroxyphenylalanine (DOPA) in an acetate buffer as their substrate.

The EEA assays employed the microplate procedures first described by Sinsabaugh and his colleagues (Sinsabaugh et al. 1997; Foreman et al. 1998; Sinsabaugh and Foreman 2001). Substrate and reference solutions were prepared in sterile deionized water and included quadruplicate assays for each enzyme and reference standards. The decrease in fluorescence due to quenching was estimated by comparing the fluorescence of the supernatant of standards mixed with soil slurries to that of the standard solution mixed only with buffer (German et al. 2011). Microplates were incubated at 22 °C for methyl-umbelliferyl-linked substrates, and at 30 °C for methyl coumarin-linked substrates. Fluorescence was measured using a Model FLX800T BioTek Instruments fluorometer with an excitation wave-length of 350 nm and an emission wavelength of 450 nm. We report ecoenzyme activity as substrate accumulated per unit soil over time. The results are adjusted for emission coefficients calculated from standards, corrected for quenching, and normalized for sample dry weight ($\text{nmol h}^{-1} \text{g}^{-1} \text{DW}$; German et al. 2011).

Ecoenzyme stoichiometry and nutrient limitation

Wetland soil C quality, microbial carbon and nutrient limitation, and CUE for each wetland soil sample were estimated from the activity and stoichiometry of the four most commonly measured hydrolase enzymes (β -glucosidase, β -N-acetylglucosaminidase, leucine aminopeptidase, and phosphatase), with phenol oxidase used to account for the effect of recalcitrant C. The wetland soil C quality index (CQI) was estimated as:

$$\text{CQI} = \ln\text{POX}/(\ln\text{BG} + \ln\text{POX}), \quad (2)$$

where $\ln\text{POX}$ and $\ln\text{BG}$, the natural log of phenol oxidase and glucosidase activities, are proxies for the relative abundances of recalcitrant C and labile C, respectively. The CQI is proportional to the amount of recalcitrant C in wetland soils. We expect the soil C pool to become more recalcitrant as organic matter decomposition progresses, resulting in slower microbial growth. We normalized our reported EEA stoichiometric ratios to POX activity to account for the decrease in the demand by the microbial assemblage for P relative to that for N to more accurately reflect relative C, N, and P limitation along the decomposition gradient (Sinsabaugh and Follstad Shah 2011):

$$\ln\text{BG}/\ln\text{POX} = \ln[\text{NAG} + \text{LAP}]/\ln\text{POX}, \quad (3)$$

$$\ln BG / \ln POX = \ln AP / \ln POX, \quad (4)$$

with deviations from a slope of 1 indicating N (Eq. 3) or P (Eq. 4) limitation relative to C acquisition.

Carbon use efficiency and organic matter decomposition

Carbon use efficiency (CUE) was estimated as a function of N and P supply relative to C availability (Sinsabaugh et al. 2013):

$$CUE = 0.6 \times (CN_S \times CP_S) / ((K_{CN} + CN_S) \times (K_{CP} + CP_S))^{0.5}, \quad (5)$$

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).

We used a microbial ecoenzyme allocation model to predict organic matter decomposition ($-k$, % day⁻¹; Sinsabaugh and Moorhead 1994):

$$-k = (CUE \times ENZ_{TOT}) / (1 + ([NAG + LAP]/BG) + (AP/BG)) \quad (6)$$

where CUE is derived from Eq. 5 and ENZ_{TOT} is the sum of all EEA normalized to their maximum values to eliminate scalar differences among the EEA. Normalized values were used for BG, [NAG + LAP], and AP in the remainder of the equation.

Statistical analyses—Each NWCA probability site has a sample weight calculated as the inverse of its selection probability (olsen et al. in press). We calculated group weighted mean ($\pm$ SE) values and percentiles for box and whisker plots using the site weights for soil chemistry and microbial variables. These weighted results make possible inference to the total population of wetlands in the continental US and not just the wetland sites that were sampled. Ecoregional and disturbance class differences in the soil and microbial variables were tested using analysis of variance, and means were compared using Tukey's Honest Significant Difference test. The stoichiometric relationships of C, N and P acquiring ecoenzyme activity were modeled using general linear regression analyses. All analyses were done using SAS for Windows, release 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results and discussion

Soil chemistry and the carbon quality index

Mean soil C ranged from 44.4 g C kg⁻¹ in the most disturbed wooded wetlands in the W ecoregion to 438 g C kg⁻¹ in least disturbed wooded wetlands in the EMU ecoregion. Mean soil N ranged from 1.32 g N kg⁻¹ in the most disturbed herbaceous wetlands in the CPL ecoregion to 32.5 g N kg⁻¹ at least disturbed herbaceous wetlands in the CPL ecoregion.

Mean soil P ranged from 0.21 g P kg⁻¹ in the most disturbed herbaceous wetlands in the CPL ecoregion to 1.32 g P kg⁻¹ in the most disturbed wooded wetlands of the W ecoregion (online Resource Table 1). Soil C and N were greater in the least disturbed CPL–H, EMU–H and EMU–W wetlands compared to the remaining ecoregion–wetland types, while soil P had no discernible pattern across ecoregion–wetland types (Table 3; Fig. 2). Nahlikand Fennessy (2016) reported similar findings from separate and deeper soil pits from these same wetlands. They attribute the greater soil C in the EMU wetlands to a cooler climate favoring organic C accumulation. Soil C and N, but not soil P, decreased with increasing wetland disturbance (Table 2; Fig. 2). This response to anthropogenic disturbances was also observed in the deeper soil pits (Nahlik and Fennessy 2016). Soil C/N ranged from 13.9/1 (intermediately disturbed IPL–H) to 31.6/1 (most disturbed W–W); soil C/P ranged from 164/1 (most disturbed WL–H) to 3283/1 (least disturbed CPL–H); and soil N/P ranged from 8.20/1 (most disturbed W–W) to 244/1 (least disturbed CPL–H; online Resource Table 1). Soil C/N, C/P and N/P were generally higher in the herbaceous and wooded CPL and EMU wetlands, and soil C/P and N/P decreased with increasing wetland disturbance (Table 2; online Resource Table 1). Mean carbon quality index (CQI) ranged from 0.46 at intermediately disturbed wooded wetlands in the IPL ecoregion to 0.58 in the most disturbed E–H wetlands, with little apparent disturbance class differences (Table 2; online Resource Table 1).

Ecoenzyme stoichiometry and nutrient limitation

Carbon acquiring EEA was dominated by β -glucosidase in all ecoregion–wetland types and across disturbance classes, followed by cellobiohydrolase, β -xylosidase, β -galactosidase, and α -galactosidase (Online Resource Table 2). In aggregate, the C acquisition enzymes varied across ecoregions and among disturbance classes, but with no consistent pattern or trend (Table 2). Nitrogen acquiring EEA was dominated by β -N-acetylglucosaminidase, followed by L-alanine aminopeptidase and L-leucine aminopeptidase, and was significantly different between ecoregion–wetland types, but with no apparent trend. N acquiring EEA did not vary among disturbance classes (Table 3; Online Resource Table 2). Phosphatase activity varied among ecoregion–wetland classes (being highest in the least disturbed EMU–W wetlands) and among disturbance classes (Table 3; Online Resource Table 2). C, N and P acquiring EEA were negatively correlated with the CQI (– 0.22 to – 0.39) and positively correlated with soil C, N and P concentrations and stoichiometry (0.15–0.64; Table 4a).

The proportion of EEA dedicated to C-acquisition ranged from 0.28 (intermediately disturbed CPL–W) to 0.76 (least disturbed W–H) and was significantly different between ecoregion–wetland types and among disturbance classes (Fig. 3a; Online Resource Table 3). N-acquisition ranged from 0.07 (least disturbed W–H) to 0.32 (most disturbed E–W) and, in most cases, increased with increasing wetland disturbance (Fig. 3b; Online Resource Table 3). P acquisition ranged from 0.13 (most disturbed IPL–W, W–H and W–W) to 0.62 (intermediately disturbed CPL–W) and varied inconsistently among wetland disturbance classes (Fig. 3c; Online Resource Table 3). The dominance of C-acquisition in many of the ecoregion–wetland types suggests that despite significant C stores in wetland soils, microbial assemblages in these soils are somewhat C-limited (Fig. 3a). The other clear message from these analyses is that wetlands are more often P- than N-limited (Fig. 3b, c),

and the P-limitation is greater in intermediate and most disturbed wetlands (Fig. 3c), suggesting that the disturbance may be related to N-loading to wetlands from agricultural and urban land uses (Nahlik and Fennessy 2016). The relative enzyme allocation toward C acquisition was positively correlated with soil C and N, and negatively correlated with the CQI (Table 4b). N acquisition was negatively correlated with soil C, soil N, and soil C/P, and positively correlated with the CQI (Table 4b). P acquisition was negatively correlated with soil P, and positively correlated with the CQI (Table 4b).

Reported rates for microbial EEA in the literature vary across several orders of magnitude, but with these emerging generalizations: (1) aquatic sediment and wetland soil ecoenzyme activity are significantly greater than EEA for terrestrial soils; and (2) EEA in all environments co-vary such that the expected enzyme ratios related to C/N/P acquisition approach unity (Sinsabaugh et al. 2009, 2012; Hill et al. 2014). These authors attributed these differences to relatively greater amounts of labile C in aquatic sediments and wetland soils compared to the more refractory C of terrestrial soils, a result of greater terrestrial soil C age and microbial processing. This model of increasing C refraction with soil age is supported by our reported inverse relationships between the activity of the glycosidases (C-acquisition) and the carbon quality index, an indicator of increasing soil C refractivity and presumably soil age.

The importance of the wetland soil carbon quality is evident by the increasing allocation of enzymes toward C acquisition, mostly at the expense of P acquisition, with increasing C recalcitrance. While wetland soils have abundant C stores (Nahlik and Fennessy 2016) and EEA stoichiometry indicative of P-limitation, a large proportion (0.28–0.76) of EEA in wetland soils is for the acquisition of C, a phenomenon previously reported for Great Lakes coastal wetlands and northern peatlands (Hill et al. 2006, 2014). The proportion of enzyme activity allocated for N acquisition (0.078–0.32) is relatively constant across ecoregion–wetland types, and across disturbance classes suggesting that N acquisition is less constraining on microbial metabolism than either C or P acquisition. This finding is in agreement with results from a global analysis of soil ecoenzyme activity and C recalcitrance, which reported that microbial C demand increases faster than N or P demand as C recalcitrance increases, indicative of the greater ecoenzymatic expenditure to acquire C as the labile C supply becomes limiting (Sinsabaugh and Follstad Shah 2011).

Microbial ecoenzyme stoichiometry was further evaluated using general linear regressions of ecoenzyme pairs normalized for phenol oxidase activity (Fig. 4; Online Resource Table 4). The slopes of the regressions lines indicate relative C-, N-, or P-limitations, with slopes > 1 indicating enhancement of the EEA on the y-axis and slopes < 1 indicating enhancement of the EEA on the x-axis. The deviation of the slope from a value of 1 is a measure of the relative degree of limitation. With few exceptions, the results of these analyses indicated an agreement among ecoregion–wetland types, and among disturbance classes about the occurrences of C, N, and P limitation of wetland microbial metabolism, with most wetlands being relatively more nutrient than carbon limited, and more P limited than N limited (Fig. 4; Online Resource Table 4).

Microbial respiration, carbon use efficiency and organic matter decomposition

Mean microbial respiration (R_m) ranged from 55.76 nmol C h⁻¹ g⁻¹ DW (most disturbed W-W) to 813 nmol C h⁻¹ g⁻¹ DW (least disturbed CPL-H; Online Resource Table 2). R_m was higher in CPL-H, EMU-H and EMU-W than in other ecoregion-wetland types, and decreased with increasing wetland disturbance (Table 2; Fig. 5a; Online Resource Table 2). R_m was positively correlated with soil C and N and with soil C/P and N/P, and negatively correlated with CQI (Table 3). Mean microbial carbon use efficiencies (CUE) ranged from 0.056 (least disturbed EMU-W) to 0.302 (intermediately disturbed CPL-H; Online Resource Table 3). CUE varied significantly among ecoregion-wetland types, but the trend is not obviously discernible. CUE increased with increasing wetland disturbance, but the trend was only marginally significant ($p = 0.0865$; Table 3; Fig. 5b; Online Resource Table 3). CUE was negatively correlated with soil C and N, and with soil C/P, and positively correlated with the CQI (Table 4b). The negative correlations with soil C and N are consistent with the reported inverse relationships between soil C quantity, C, N and P stoichiometry, and microbial carbon use efficiency (Manzoni et al. 2012; Sinsabaugh et al. 2013). Organic matter decomposition rates ($-k$) were slowest (0.011% day⁻¹) in the most disturbed E-H wetlands and fastest (0.048% day⁻¹) in the most disturbed IPL-H wetlands (Online Resource Table 3). $-k$ was slower in the estuarine (E-H and E-W) wetlands than in wetlands from the other ecoregions, and varied significantly among disturbance classes, but with no discernible trend (Table 3; Fig. 5c; Online Resource Table 3). $-k$ was negatively correlated with soil C/N and CQI, and positively correlated with soil C, N, and P, highlighting the stoichiometric demands of microbial metabolism related to soil organic matter decomposition (Table 4b).

Enzyme decomposition models (EDM) integrate microbial EEA related to C and nutrient acquisition with environmental pools of C and nutrients to predict organic matter decomposition rates (Sinsabaugh and Moorhead 1994; Schimel and Weintraub 2003; Moorhead et al. 2012). Our national, ecoregional, and disturbance class estimates of wetland organic matter decomposition based on EEA compare favorably with rates from a variety of more localized wetland studies (Brinson et al. 1981; Jackson et al. 1995; Alvarez and Guerrero 2000; Rejmankova and Houdkova 2006; Hill et al. 2014), and demonstrate the scalability of EDM from the site to the national scale.

The vast stores of C in wetland soils are largely the result of hundreds to thousands of years of wetland primary production exceeding respiration (Mitra et al. 2005; Bridgham et al. 2006; Nahlik and Fennessy 2016). While wetlands continue to accumulate C at globally averaged rates of 200–3200 kg ha⁻¹ year⁻¹ (Mitra et al. 2005; Bridgham et al. 2006; Kayranli et al. 2010; Mitsch et al. 2013), their greatest potential impact on the global C budget and climate may be as a source of C to the atmosphere upon decomposition of this accumulated wetland soil organic matter (Gorham 1991; Mitra et al. 2005; Bridgham et al. 2006; Mitsch et al. 2013; Hill et al. 2014; Nahlik and Fennessy 2016). Our measured respiration rates and modeled decomposition rates indicate significant C losses from wetlands across the conterminous US which may increase with rising temperatures and lowering water tables, but which may also be constrained by C, N, or P availability. Microbial respiration, EEA, and decomposition rates were strongly correlated with soil C, N

and P pools, suggesting a more localized control of elemental acquisition to support microbial biomass maintenance and accumulation. The implications of a localized control are 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, resulting in no discernible change in C storage in US wetland landscapes (Bridgham et al. 2006; Hill et al. 2014).

Conclusions

This study represents a unique assessment of microbial enzyme activities, microbial respiration, and organic matter decomposition in wetland sediments across broad ecoregional and national scales. With the exception of herbaceous and wooded wetlands of the EMU ecoregion, a region noteworthy for its extent of wetlands and its cooler climate, wetlands across the conterminous United States had surprisingly similar surface layer (0–10 cm depth) stores of soil C, N and P. The data also show that wetland stores of C and N are inversely related to the magnitude of human disturbances. Wetland soil chemistries were correlated with microbial respiration and with indicators of C, N, and P limitation. Our data also indicated that wetland soil C quality was similar across ecoregion–wetland types and disturbance classes, and that microbial use of this C, and the processing of organic matter, was similarly constrained by C and nutrient availability. Our results from the enzyme-based approach for modeling C, N, and P acquisition, and organic matter decomposition, all rooted in stoichiometric theory, suggests that this is a valid approach for modeling resource limitations of microbial metabolism and biogeochemical cycling in wetlands. Given the ease of collecting and analyzing soil EEA and their response to wetland disturbance gradients, enzyme stoichiometry models are 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Map of the conterminous United States with the four ecoregions delineated and with the locations and disturbance class status of the 792 National Wetland Condition Assessment sites from which microbial enzyme samples were analyzed in 2011. Estuaries along the coasts formed a fifth ecoregion (EE, see text) that was too narrow to map as a shaded area

Fig. 2.

Box and whisker plots for **a** soil total carbon concentration, **b** soil total nitrogen concentration, and **c** soil total phosphorus concentration by ecoregion–wetland type. Boxes represent the 25th and 75th percentiles; the bar across each box is its median value; the symbol associated with each bar is the mean; and the whiskers represent the minimum and maximum values. The first bar in each cluster is for least disturbed wetlands (light gray bar), followed by intermediately disturbed wetlands (medium gray), and most disturbed wetlands (dark gray). Ecoregion–wetland type abbreviations: herbaceous and wooded coastal plains

(CPL–H/W); eastern mountains and upper midwest (EMU–H/W); interior plains (IPL–H/W); west (W–H/W) and herbaceous (E–H) and wooded (E–W) estuarine sites from the Atlantic, Gulf of Mexico, and Pacific coasts

Fig. 3.

Box and whisker plots for the proportion of microbial enzyme activity allocated for **a** carbon acquisition, **b** nitrogen acquisition, and **c** phosphorus acquisition by ecoregion–wetland type. Boxes represent the 25th and 75th percentiles; the bar across each box is its median value; the symbol associated with each bar is the mean; and the whiskers represent the minimum and maximum values. The first bar in each cluster is for least disturbed wetlands (light gray bar), followed by intermediately disturbed wetlands (medium gray), and most disturbed wetlands (dark gray). Ecoregion–wetland type abbreviations: herbaceous and wooded

coastal plains (CPL–H/W); eastern mountains and upper midwest (EMU–H/W); interior plains (IPL–H/W); west (W–H/W) and herbaceous (E–H) and wooded (E–W) estuarine sites from the Atlantic, Gulf of Mexico, and Pacific coasts

Fig. 4. POX-normalized ecoenzyme stoichiometry of **a** $\blacktriangleright$ acquisition relative to N acquisition; **b** C acquisition relative to P acquisition; and **c** N acquisition relative to P acquisition in US wetlands. Least disturbed wetlands are indicated by +; intermediately disturbed wetlands by O; and most disturbed wetlands by X. The dashed gray lines represent the 1:1 line, above which represents reduced ecoenzyme activity of the x-axis variable relative to the y-axis variable and below which represents reduced ecoenzyme activity of the y-axis variable relative to the x-axis variable. Model results are presented in Online Resource Table 4

Fig. 5.

Box and whisker plots for **a** microbial respiration, **b** microbial carbon use efficiency, and **c** organic matter decomposition rates ecoregion-wetland type. Boxes represent the 25th and 75th percentiles; the bar across each box is its median value; the symbol associated with each bar is the mean; and the whiskers represent the minimum and maximum values. The first bar in each cluster is for least disturbed wetlands (light gray bar), followed by intermediately disturbed wetlands (medium gray), and most disturbed wetlands (dark gray). Ecoregion-wetland type abbreviations: herbaceous and wooded coastal plains (CPL-H/W);

eastern mountains and upper midwest (EMU-H/W); interior plains (IPL-H/W); west (W-H/W) and herbaceous (E-H) and wooded (E-W) estuarine sites from the Atlantic, Gulf of Mexico, and Pacific coasts

Table 1

Lists of (a) the combined ecoregions and wetland types, number of NWCA probability sites sampled (n), and their wetland areas; and (b) ecoenzymes, their full names and EC numbers

Ecoregion-wetland type	Acronym	n	Wetland area (km ²)
(a)			
Coastal plains-herbaceous	CPL-H ^a	51	12,749
Coastal plains-woody	CPL-W ^b	147	84,281
Eastern mountains and upper midwest-herbaceous	EMU-H ^a	51	13,641
Eastern mountains and upper midwest-woody	EMU-W ^b	71	57,200
Interior plains-herbaceous	IPL-H ^a	66	10,754
Interior plains-woody	IPL-W ^b	36	11,908
West-herbaceous	W-H ^a	63	5854
West-woody	W-W ^b	43	6365
Estuarine-herbaceous	E-H ^c	199	12,722
Estuarine-woody	E-W ^d	65	2006
Survey total		792	217,480
(b)			
Enzyme name	Acronym	Function	EC number
Dehydrogenase	DHA	Respiratory electron transport system	
α-D-Galactosidase	AGAL	C acquisition	3.2.1.22
β-D-Galactosidase	BGAL	C acquisition	3.2.1.23
β-D-Glucosidase	BG	C acquisition	3.2.1.21
Cellobiohydrolase	CBH	C acquisition	3.2.1.91
β-D-Xylosidase	BX	C acquisition	3.2.1.8
β-N-acetylglucosaminidase	NAG	N acquisition	3.2.1.50
L-Alanine aminopeptidase	AAP	N acquisition	3.4.11.2
L-Leucine aminopeptidase	LAP	N acquisition	3.4.11.1

Enzyme name	Acronym	Function	EC number
Acid phosphatase	AP	P acquisition	3.1.3.2
Polyphenol oxidase	POX	C acquisition	1.14.18.1

^aH = Emergent, ponded, or previously farmed wetlands in palustrine, shallow riverine, or shallow lacustrine littoral settings

^bW = Forest or shrub dominated wetlands in palustrine, shallow riverine, or shallow lacustrine littoral settings

^cH = Estuarine emergent wetlands

^dW = Estuarine shrub or forested wetlands

Table 2

Summary statistics of the type I general linear model analysis of an unbalanced, nested (ecoregion–wetland type, condition class, and their interaction) sampling design for the means for soil TC, TN, TP (g kg⁻¹), soil C/N, C/P, and N/P (molar); and the soil carbon quality index (CQI) in wetlands of the continental United States

Variable	Effects	df	F	P
Soil TC	Ecuregion–wetland type	9	7.82	< 0.0001
	Condition class	2	12.73	< 0.0001
	Ecuregion–wetland type (condition)	18	2.23	0.0024
Soil TN	Ecuregion–wetland type	9	47.61	< 0.0001
	Condition class	2	19.04	< 0.0001
	Ecuregion–wetland type (condition)	18	6.68	< 0.0001
Soil TP	Ecuregion–wetland type	9	21.67	< 0.0001
	Condition class	2	6.77	0.0012
	Ecuregion–wetland type (condition)	18	4.18	< 0.0001
Soil C/N	Ecuregion–wetland type	9	9.15	< 0.0001
	Condition class	2	3.24	0.0397
	Ecuregion–wetland type (condition)	18	1.97	0.0094
Soil C/P	Ecuregion–wetland type	9	10.18	< 0.0001
	Condition class	2	16.05	< 0.0001
	Ecuregion–wetland type (condition)	18	3.13	< 0.0001
Soil N/P	Ecuregion–wetland type	9	7.89	< 0.0001
	Condition class	2	7.20	0.0008
	Ecuregion–wetland type (condition)	18	3.52	< 0.0001
CQI	Ecuregion–wetland type	9	14.59	0.0026
	Condition class	2	0.32	0.7299
	Ecuregion–wetland type (condition)	18	2.23	0.0025

All variables are natural log transformed before analysis

Table 3

Summary statistics of the type I general linear model analysis of an unbalanced, nested (ecoregion–wetland type, condition class) sampling design for the means for microbial respiration (R_m , $\text{nmol C h}^{-1} \text{g}^{-1} \text{DW}$), proportion of enzyme activity devoted to C, N, and P acquisition (%), carbon use efficiency (CUE), and organic matter decomposition ($-k$, $\% \text{ day}^{-1}$), in wetlands of the continental United States

Variable	Effects	df	F	P
Rm	Ecoregion–wetland type	9	14.13	< 0.0001
	Condition class	2	9.71	< 0.0001
	Ecoregion–wetland type (condition)	18	1.20	0.2576
C acquisition	Ecoregion–wetland type	9	69.93	< 0.0001
	Condition class	2	4.08	0.0173
	Ecoregion–wetland type (condition)	18	3.68	< 0.0001
N acquisition	Ecoregion–wetland type	9	15.41	< 0.0001
	Condition class	2	1.88	0.1536
	Ecoregion–wetland type (condition)	18	3.27	< 0.0001
P acquisition	Ecoregion–wetland type	9	59.44	< 0.0001
	Condition class	2	5.20	0.0057
	Ecoregion–wetland type (condition)	18	5.33	< 0.0001
CUE	Ecoregion–wetland type	9	33.04	< 0.0001
	Condition class	2	2.46	0.0865
	Ecoregion–wetland type (condition)	18	2.66	0.0002
$-k$	Ecoregion–wetland type	9	3.58	0.0002
	Condition class	2	10.53	< 0.0001
	Ecoregion–wetland type (condition)	18	4.20	< 0.0001

All variables are natural log transformed before analysis

Table 4

Spearman rank correlation coefficients for (a) microbial respiration (R_m) and EEA, and (b) microbial C, N, and P acquisition, carbon use efficiency (CUE), and organic matter decomposition ($-k$) with soil chemistry and the carbon quality index (CQI) for US wetlands

	C acquisition				N acquisition				P acquisition	
	R_m	AGAL	BGAL	BG	CBH	BX	NAG	AAP	LAP	AP
(a)										
Soil chemistry										
TC	0.64	0.60	0.62	0.62	0.48	0.50	0.27	0.48	0.41	0.47
TN	0.48	0.53	0.64	0.58	0.45	0.42	0.23	0.47	0.39	0.43
TP	-	-	0.19	0.17	0.15	-	-	0.21	-	-
Soil stoichiometry										
C/N	0.34	0.18	-	-	-	0.19	-	-	-	0.18
C/P	0.48	0.28	0.26	0.32	0.21	0.27	-	0.20	0.17	0.26
N/P	0.22	0.15	0.18	0.17	-	-	-	-	-	-
Carbon quality index										
CQI	-0.26	-0.32	-0.31	NA	-0.39	-0.29	-0.33	-0.26	-0.22	-0.29
(b)										
Soil chemistry										
TC		0.19	-0.31	-	-0.61	0.21				
TN		0.24	-0.30	-	-0.56	0.29				
TP		-	-	-0.16	-	0.33				
Soil stoichiometry C/N										
C/P		-	-0.20	-	-0.48	-				
N/P		-	-	-	-0.30	-				
Carbon quality index CQI										
		-0.41	0.31	0.29	0.53	-0.25				

Reported correlation coefficients are significant ($p < 0.05$ and $r > 0.15$). Non-significant correlations are indicated by dash (-). Full descriptions of the EEA are given in Table 1b