RESEARCH ARTICLE

Key Points:
- Estimates of watershed C and N cycling rates were higher with this approach
- B horizons accounted for over half of C pools and microbial biomass C
- Surface horizons dominated N-related variables across hydropedologic settings

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Searching for biogeochemical hot spots in three dimensions: Soil C and N cycling in hydropedologic settings in a northern hardwood forest

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Abstract Understanding and predicting the extent, location, and function of biogeochemical hot spots at the watershed scale is a frontier in environmental science. We applied a hydropedologic approach to identify (1) biogeochemical differences among morphologically distinct hydropedologic settings and (2) hot spots of microbial carbon (C) and nitrogen (N) cycling activity in a northern hardwood forest in Hubbard Brook Experimental Forest, New Hampshire, USA. We assessed variables related to C and N cycling in spodic hydropedologic settings (typical podzols, bimodal podzols, and Bh podzols) and groundwater seeps during August 2010. We found that soil horizons (Oi/Oe, Oa/A, and B) differed significantly for most variables. B horizons (>10 cm) accounted for 71% ($\pm11\%$) of C pools and 62% ($\pm10\%$) of microbial biomass C in the sampled soil profile, whereas the surface horizons (Oi/Oe and Oa/A; 0–10 cm) were dominant zones for N-cycle-related variables. Watershed-wide estimates of C and N cycling were higher by 34 to 43% (±17–19%) when rates, horizon thickness, and areal extent of each hydropedologic setting were incorporated, versus conventionally calculated estimates for typical podzols that included only the top 10 cm of mineral soil. Despite the variation in profile development in typical, bimodal, and Bh podzols, we did not detect significant differences in C and N cycling among them. Across all soil horizons and hydropedologic settings, we found strong links between biogeochemical cycling and soil C, suggesting that the accumulation of C in soils may be a robust indicator of microbial C and N cycling capacity in the landscape.

1. Introduction

Although biogeochemical variability occurs at multiple spatial scales, there has long been considerable interest in understanding variability at the watershed scale for both research and management purposes [*McGuire and Likens*, 2011]. Studies of linkages between watershed hydrology and biogeochemistry have been productive [*Burt and Pinay*, 2005; *Lohse et al.*, 2009], integrating surface and subsurface processes and yielding conceptual advances, such as simulation models of watershed carbon (C) and nitrogen (N) fluxes [*Band et al.*, 2001], ways to estimate and map soil C pools and microbial processes [*Cosandey et al.*, 2003; *Webster et al.*, 2011], and recognition of biogeochemical hot spots in the landscape [*Burt and Pinay*, 2005; *McClain et al.*, 2003].

Biogeochemical hot spots are zones where reaction rates are disproportionately higher than in the surrounding matrix and are often found where hydrologic flowpaths converge and bring reactants together [*Burt et al.*, 2010; *McClain et al.*, 2003]. Identifying biogeochemical hot spots in the landscape has proven a fruitful direction of research, particularly for processes such as denitrification (microbial conversion of nitrate (NO_3^-) to gaseous nitrous oxide (N_2O) or dinitrogen (N_2) [*Knowles*, 1982]) that rely on anoxic zones and multiple substrates such as NO_3^- and labile C [*Gold et al.*, 1998; *Harms and Grimm*, 2008]. However, the significance of hot spots at the watershed scale depends on the magnitude of transport and reaction rates in hot spots and their spatial extent [*Ocampo et al.*, 2006], relative to the lower rates but greater extent represented by the surrounding matrix [*McClain et al.*, 2003].

Investigations of C and N biogeochemistry in forest soils typically focus on the top 10–15 cm of soil, as this is generally the most biologically active zone, but incorporating deeper soils into biogeochemical studies is

important for several reasons: (1) deeper soils generally have lower biogeochemical processing rates per kilogram of soil and are often below the rooting zone, yet they represent a potentially significant but often unmeasured component of landscape C and N pools and fluxes [*Harrison et al.*, 2011; *Jobbágy and Jackson*, 2000, 2001; *Rumpel and Kogel-Knabner*, 2011] due to the large but variable volume they occupy in watersheds [*Richter and Markewitz*, 1995]; (2) deeper soils where subsurface hydrologic flowpaths converge may represent biogeochemical hot spots that rival the process rates found in surface soils [*McClain et al.*, 2003]; and (3) without quantifying the contribution of deeper soils to C and N cycling and pools, we may be underestimating C and N biogeochemistry at the landscape scale. As a result of this often overlooked heterogeneity in soil structure and processes, accurately integrating the function of biogeochemical hot spots with the surrounding matrix at the watershed scale using empirical and modeling methods has been difficult [*Groffman et al.*, 2009] and remains a frontier in environmental science [*National Research Council*, 2001; *Sommer*, 2006].

One promising move toward better prediction of soil variability in the landscape is the emerging discipline of hydropedology, which brings together soil physics, hydrology, and pedology [*Lin*, 2003]. Hydrologic flowpaths are a key element of the hydropedologic framework, because soil development occurs laterally along connected hydrologic flowpaths as well as vertically through soil profiles [*Lohse and Dietrich*, 2005; *Sommer et al.*, 2000]. Over time, structurally and functionally distinct hydropedologic settings can form in the landscape along predictable sequences driven by surface and subsurface topography and hydrology through processes such as podzolization [*Sommer et al.*, 2000]. Predicting the location, extent, and functioning of distinct hydropedologic settings in the landscape is potentially of great utility for modeling and upscaling biogeochemical and hydrologic processes [*Sommer*, 2006].

This hydropedologic approach has been applied at the Hubbard Brook Experimental Forest Long Term Ecological Research site in New Hampshire, USA. Five distinct hydropedologic settings have been identified in Watershed 3, a hydrologic reference watershed, based on morphology and landscape position, within the general Spodosol soil type that has been previously described at the Hubbard Brook Experimental Forest (HBEF) [*Huntington et al.*, 1988; *Johnson et al.*, 2000]. In our classification scheme, E podzols and Bhs podzols are found near the watershed divide where bedrock is near the surface and lateral flowpaths appear to be the dominant mechanisms of horizon development [*Bailey et al.*, 2014]. The first hydropedologic setting, E podzols, has thicker E horizons (eluviated mineral soils with low organic matter and nutrient content) and generally lacks B horizons (mineral soils with accumulations of clay, iron and aluminum oxides, or organic matter) [see *Sommer et al.*, 2000]. The second hydropedologic setting, Bhs podzols, tends to lack an E horizon and has a thick Bhs horizon (accumulation of organic matter with aluminum and iron oxides). Midslope positions tend to feature typical podzols, the third and dominant hydropedologic setting in the watershed, with thin E and Bhs horizons over a thicker Bs horizon (but not a Bh horizon, accumulation of organic matter). Bimodal podzols, the fourth type, are found at the transition toward zones of accumulation, such as footslopes, and have a thin Bh horizon below a typical podzol E-Bhs-Bs sequence. The fifth spodic hydropedologic setting, the Bh podzols, has a dark Bh horizon, usually no E horizon, and tends to be found on lower slopes, such as benches and riparian zones. Seep Inceptisols (Aquepts), with light-colored Bw/Bg horizons (evidence of anaerobic conditions), in contrast to the spodic hydropedologic settings, occur where the groundwater seeps to the surface [*Bailey et al.*, 2014]. Within this hydropedologic framework, zones of C accumulation such as the Bh and Bhs horizons may represent biogeochemical hot spots in the watershed.

Our objectives were (1) to compare soil C and N cycling rates and pools in four hydropedologic settings (three distinct spodic hydropedologic settings and seeps) in a northern hardwood forested catchment at the height of the growing season, (2) to determine whether any of these hydropedologic settings represented biogeochemical hot spots in the landscape, and (3) to assess the importance of different hydropedologic settings and deeper soil horizons to landscape-scale estimates of biogeochemical cycling. We assessed these variables on a mass basis (i.e., per gram of soil) as well as on an areal basis (i.e., per square meter of land surface). We expected to find that the Bh podzols, as zones in the landscape where hydrologic flowpaths and reactants converge, might have higher biogeochemical activity compared to the upslope hydropedologic settings (e.g., typical podzols) and that the B horizons in Bh podzols would therefore contribute disproportionately to the biogeochemical activity of the soil profile.
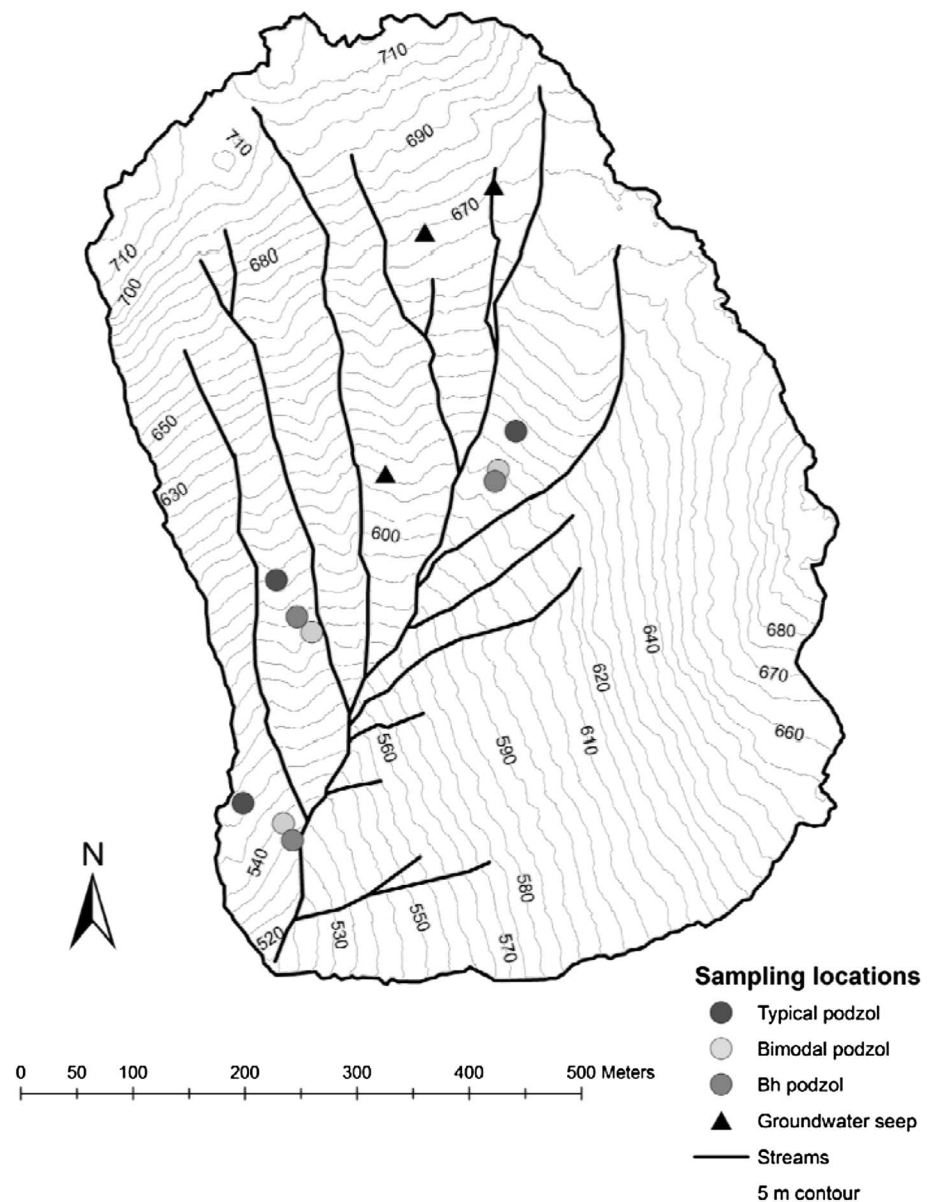
Figure 1. Map of Watershed 3, with soil sampling locations shown by hydropedologic setting.

2. Methods

2.1. Site Description and Field Sampling Design

We conducted this study in a 42 ha reference watershed (WS3) at the Hubbard Brook Experimental Forest (HBEF) in the White Mountain National Forest in central New Hampshire, USA (43°56′ N, 71°45′W). The region experiences a humid continental climate (mean monthly temperatures range from −9 to 18°C; annual precipitation is 1400 mm). This northern hardwood forest is dominated by American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), and yellow birch (*Betula allegheniensis*) with upper elevations and shallow-to-bedrock areas dominated by conifers (red spruce and balsam fir) interspersed with mountain white birch [*Bohlen et al.*, 2001].

In August 2010, we selected three hillslope transects in WS3, each with three sampling sites that have been well characterized in ongoing hydropedologic studies of soil profile characteristics [e.g., *Bailey et al.*, 2014] and soil water and gas dynamics [e.g., *Werner et al.*, 2011]. In addition, we sampled three groundwater seeps in the watershed for inclusion in our study. We sampled four types of hydropedologic settings: three spodic hydropedologic settings (typical podzols, bimodal podzols, and Bh podzols) and groundwater seeps (Figure 1).
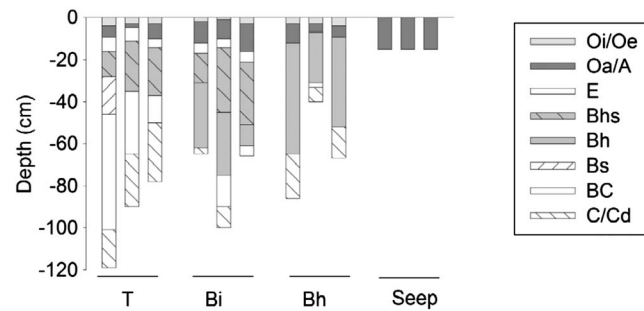
Figure 2. Sampled depth profiles by soil horizon and hydropedologic setting (T: typical podzol, Bi: bimodal podzol, and Bh: Bh podzol). Horizons shown in white (E, Bs, BC, C, and Cd) were not analyzed in this study.

We dug soil pits with a shovel and used a hand trowel to collect grab samples from the center of each horizon (Oi/Oe (forest floor), Oa/A, and B) at each hillslope sampling site to a maximum pit depth of 76 cm. We also collected 2–3 samples of surface soils (0–15 cm) at the groundwater seep sites, resulting in a total of 37 individual soil samples (Figure 2).

2.2. Laboratory Methods

Samples were stored at 4°C from the time of collection until analysis within 3 weeks. Roots and rocks were removed by hand during sample homogenization. Analyses were performed on samples at field moisture. Soil moisture at the time of sampling was determined by oven-drying samples for 48 h at 60°C. Soil C content was determined by elemental analysis on a Carlo Erba NC2500 elemental analyzer at Cornell University Stable Isotope Laboratory.

We determined inorganic N content by extracting 7.5 g of soil with 30 mL of 2 M potassium chloride (KCl) for 1 h on a shaker table at 125 rpm, filtering the extract through Whatman 42 filter paper, and analyzing the extracts for ammonium (NH_4^+) and nitrate (NO_3^-) by colorimetric analysis with a Quikchem 8100 flow injection analyzer (Lachat Instruments, Milwaukee, WI), using the cadmium reduction method (10-107-04-1-B) for NO_3-N and the sodium salicylate method (12-107-06-2-A) for NH_4-N.

Soil microbial biomass C and N content was estimated using the chloroform fumigation-incubation method, in which soils are fumigated with chloroform to lyse microbial cells, then inoculated with 0.2 g fresh soil and incubated for 9 days at room temperature [*Paul et al.*, 1999]. The carbon dioxide (CO_2) released by actively growing microbes and the net change in inorganic N are assumed to be proportional to the C and N content of the original microbial biomass in the sample. To calculate the microbial biomass C from the CO_2 produced during the incubation, we used a proportionality constant ($kc = 0.41$), but no proportionality constant was used for microbial biomass N [*Paul et al.*, 1999].

We estimated potential microbial respiration, potential net nitrification, and potential N mineralization by measuring CO_2 production (by gas chromatography with thermal conductivity detection) and inorganic N release (soils extracted for N analysis as described above) from unfumigated control samples in parallel 9 day incubations [*Robertson et al.*, 1999]. The accumulation of NH_4^+ and NO_3^- represented potential net N mineralization, while NO_3^- alone represented potential net nitrification. The production of CO_2 over 9 days represented potential microbial respiration.

We performed the denitrification enzyme activity assay, in which 5 g soil samples are incubated under anaerobic conditions with NO_3^-, glucose, chloramphenicol, and acetylene; the net production of N_2O between 30 and 90 min as analyzed by gas chromatography with electron capture detection is an estimate of potential denitrification [*Groffman et al.*, 1999].

2.3. Data Analysis

To determine if statistical differences existed by hydropedologic setting and by soil horizon, we initially analyzed results per gram of soil. In order to convert rates on a mass basis to areal basis, bulk density for each soil sample was needed. We estimated the bulk density (BD) of the <2 mm fraction of each soil sample from its C content, based on an empirical method developed for forest soils in the northeast U.S. region [*Federer et al.*, 1993] that was validated using 34 soil samples in this watershed (BD = 1.40 + −0.756 log (%C), $r^2 = 0.817$ [*Bailey et al.*, 2014]). From the calculated BD and measured horizon thickness for each sample, we estimated areal quantities and the process rates of the variables we measured for each profile. By summing the soil horizons for each soil pit, we were able to compare the potential rates of C and N cycling of the four hydropedologic settings.

We applied permutational analysis of variance [*Wheeler*, 2010], a form of nonparametric analysis of variance, and adjusted P values for multiple comparisons between groups [*Hothorn et al.*, 2008] to determine whether the means of hydropedologic settings or soil horizons were different, thus avoiding the requirements of normal distributions and larger sample sizes. These analyses were performed with R statistical software ([*R Development Core Team*, 2009] R package lmPerm [*Wheeler*, 2010] and R package coin [*Hothorn et al.*, 2008]). We used linear, exponential, and Gaussian regression models to determine the best fit curves to show the variation in biogeochemical variables as a function of C content across soil horizons (SigmaPlot v11).

We used results from a survey of 58 soil pits in Watershed 3 over multiple years [*Bailey et al.*, 2014] to more robustly estimate the soil horizon thickness by hydropedologic setting in order to scale up our estimates of C and N cycling rates to the watershed. Field surveys, field observations, and 5 m digital elevation models derived from light detection and ranging were compiled in a geographic information system (GIS) to produce estimates of coverage by hydropedologic setting in the watershed [*Gillin*, 2013]. These values were used to produce area-weighted extrapolations of biogeochemical process rates at the watershed scale. We calculated mean rates from summertime measurements (1996–2012) at a nearby watershed in HBEF to compare to our results, which we upscaled in three different ways. To estimate the significance of deeper (>10 cm) soil horizons to the total soil profile biogeochemistry, we calculated the estimates of C and N cycling rates per square meter and across the 42 ha watershed, using (1) the mean areal rates for the dominant soil type (typical podzol) and only the top 10 cm of B horizons (a conventional method of upscaling) and (2) the areal rates and profile thicknesses specific to each hydropedologic setting, along with estimates of coverage in the watershed. For the purposes of this extrapolation, hydropedologic settings that were unmeasured in this study (E podzols and Bhs podzols) were conservatively assumed to have the same biogeochemical process rates as the dominant typical podzol but distinct soil profiles based on previous descriptions [*Bailey et al.*, 2014]. Biogeochemical process rates at the watershed scale, for both the mean of data from 1996–2012 and for Watershed 3, were area weighted to account for bedrock outcrops representing $9 \pm 1\%$ of the watershed area. Error propagation analysis was performed throughout the study to calculate the combined uncertainty (U_c) due to random variation (U_a; standard error of the mean) and to measurement errors (U_b); U_c is the geometric mean of U_a and U_b [*Jeter*, 2005].

3. Results

The three spodic hydropedologic settings we sampled were significantly different in terms of overall profile depths (typical podzols: 96 ± 12 cm; bimodal podzols: 77 ± 11 cm; Bh podzols: 64 ± 13 cm; and $P = 0.028$), but Oi/Oe horizons (forest floor) had uniform depths across hydropedologic settings (2.0 ± 0.6 cm to 3.3 ± 0.3 cm; Figure 2). The Oa/A horizons were significantly different across the spodic hydropedologic settings ($P = 0.073$), such that they were generally thicker in bimodal podzols (10.7 ± 1.2 cm) compared to typical podzols and Bh podzols (4.7 ± 1.5 cm and 6.0 ± 1.5 cm, respectively). Although Bhs horizons were absent in the Bh podzol hydropedologic setting (Figure 2), bimodal podzols (48.7 ± 6.3 cm) and Bh podzols (40.0 ± 8.5 cm) having generally thicker B horizons compared to typical podzols (25.7 ± 2.2 cm). The E horizon was absent in Bh podzols, and seeps had no Oi/Oe horizon development (Figure 2).

General soil characteristics, such as gravimetric water content, %C, NH_4^+, NO_3^-, and microbial biomass C and N per gram of soil did not differ significantly by hydropedologic setting, but all variables were significantly different by soil horizon ($P < 0.05$). Measured values were generally lower in B horizons compared to Oi/Oe and Oa/A horizons (Table 1).

Likewise, significant differences were found by soil horizon ($P < 0.0017$) but not by hydropedologic setting for C and N cycling variables (potential microbial respiration, potential net N mineralization, potential net nitrification, and denitrification potential) on a mass basis (Figure 3). Per gram of soil, the B horizons had significantly ($P < 0.05$) lower rates of C and N cycling compared to other horizons (Figure 3). Seep Oa/A horizons were significantly different from spodic Oa/A horizons in terms of water content, NO_3^- pools, potential net N mineralization rates, and potential net nitrification rates calculated on a mass basis (Table 1 and Figure 3).

Table 1. Gravimetric Soil Moisture, C Content, KCl-Extractable Inorganic N, and Microbial Biomass C (MBC) and N (MBN) by Hydropedologic Setting (HPS) and Soil Horizon, per Gram of Dry Soil[a]

| HPS	Horizon	n	% Moisture	%C	NH$_4$-N (µg/g soil)	NO$_3$-N (µg/g soil)	MBC (mg C/g soil)	MBN (mg N/g soil)
T	Oi/Oe	3	78.3 ± 0.70*	50.6 ± 0.78*	152 ± 33*	16.8 ± 10*	4.78 ± 1.1*	0.774 ± 0.038*
	Oa/A	3	61.1 ± 11**	32.9 ± 12**	30.4 ± 28**	18.3 ± 6.5*	2.32 ± 1.2**	0.320 ± 0.12**
	B	3	38.0 ± 5.5**	7.40 ± 1.7***	2.40 ± 1.0***	3.33 ± 1.7**	0.481 ± 0.20***	0.0324 ± 0.021***
Bi	Oi/Oe	3	71.3 ± 1.7*	37.8 ± 3.6*	83.7 ± 27*	9.60 ± 2.5*	6.21 ± 0.59*	0.511 ± 0.041*
	Oa/A	3	51.7 ± 3.0**	15.8 ± 1.9**	6.7 ± 3.0**	9.97 ± 1.5*	1.30 ± 0.39**	0.226 ± 0.052**
	B	3	36.6 ± 2.8**	5.60 ± 0.78***	2.12 ± 0.3***	0.530 ± 0.33**	0.275 ± 0.064***	0.0216 ± 0.010***
Bh	Oi/Oe	3	76.6 ± 4.7*	44.3 ± 7.7*	144 ± 50*	17.8 ± 17*	8.23 ± 0.42*	0.710 ± 0.062*
	Oa/A	3	64.0 ± 4.8**	23.4 ± 3.0**	4.93 ± 2.9**	15.9 ± 4.7*	3.31 ± 1.5**	0.333 ± 0.054**
	B	3	39.7 ± 6.4**	6.00 ± 1.6***	1.46 ± 0.2***	2.67 ± 0.33**	0.569 ± 0.071***	0.036 ± 0.010***
Seep	Oa/A	3	80.9 ± 1.6*	23.2 ± 6.3**	9.18 ± 2.0**	1.02 ± 0.60**	5.06 ± 2.2**	0.238 ± 0.064**

[a]There were no statistical differences between hydropedologic settings; differences in means ($P < 0.1$) between soil horizons are indicated by different asterisks. (T: typical podzols, Bi: bimodal podzols, and Bh: Bh podzols.)

The areal estimates of several variables differed significantly by hydropedologic setting (soil moisture ($P = 0.014$), C pools ($P = 0.037$), and NO$_3$$^-$ pools ($P = 0.060$); Figure 4). Patterns of variation were inconsistent among spodic hydropedologic settings, but seeps tended to have lower values compared to bimodal podzols and Bh podzols, except for microbial biomass C and N (Figure 4). With respect to C and N cycling processes, seeps had significantly lower potential net N mineralization ($P = 0.024$) and nitrification ($P = 0.019$) compared to Bh podzols (Figure 5). Microbial respiration and denitrification potential were not significantly different across the four sampling locations. Among the spodic hydropedologic settings, B horizons dominated the soil profile in terms of water content, C content, microbial biomass C, and microbial respiration, while the Oa/A horizon contributed the most to microbial biomass N, net nitrification, and denitrification potential (Figures 4 and 5). The forest floor (Oi/Oe) accounted for the largest pool of NH$_4$$^+$ overall (Figures 4 and 5).

Measured biogeochemical variables and processes varied consistently and significantly as a function of soil C concentration, often with clear separation between soil horizons (Figure 6). Water content varied significantly with soil C concentration, but with different relationships in spodic hydropedologic settings ($r^2 = 0.90$) and in groundwater seeps ($r^2 = 0.62$). Carbon concentration was a strong predictor of microbial biomass N ($r^2 = 0.84$),
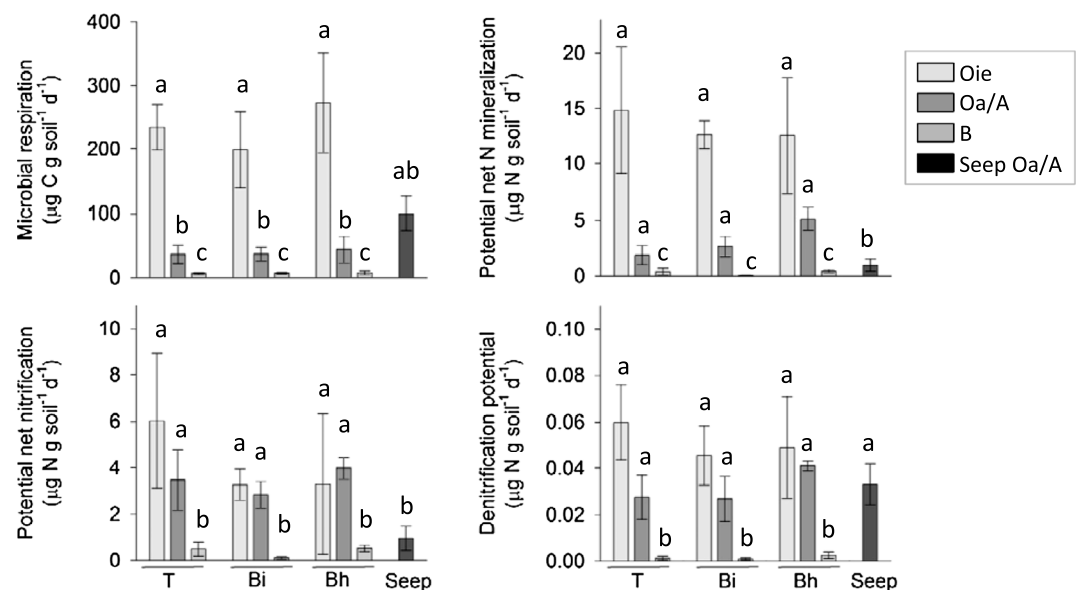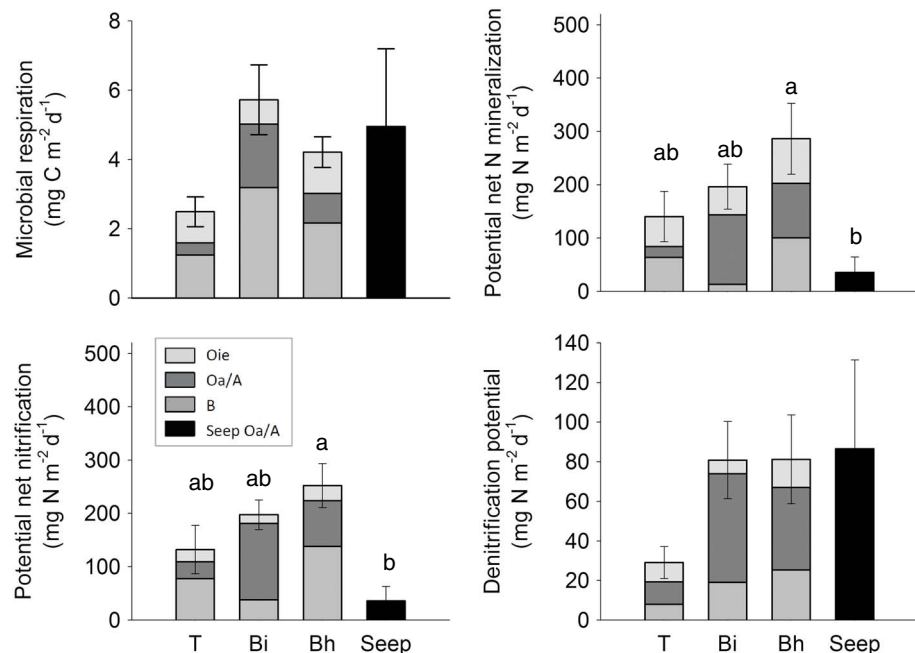

Figure 3. Soil C and N cycling variables (mass basis) by soil horizon and hydropedologic setting (T: typical podzol, Bi: bimodal podzol, and Bh: Bh podzol). Different letters indicate significant differences at $P < 0.10$.
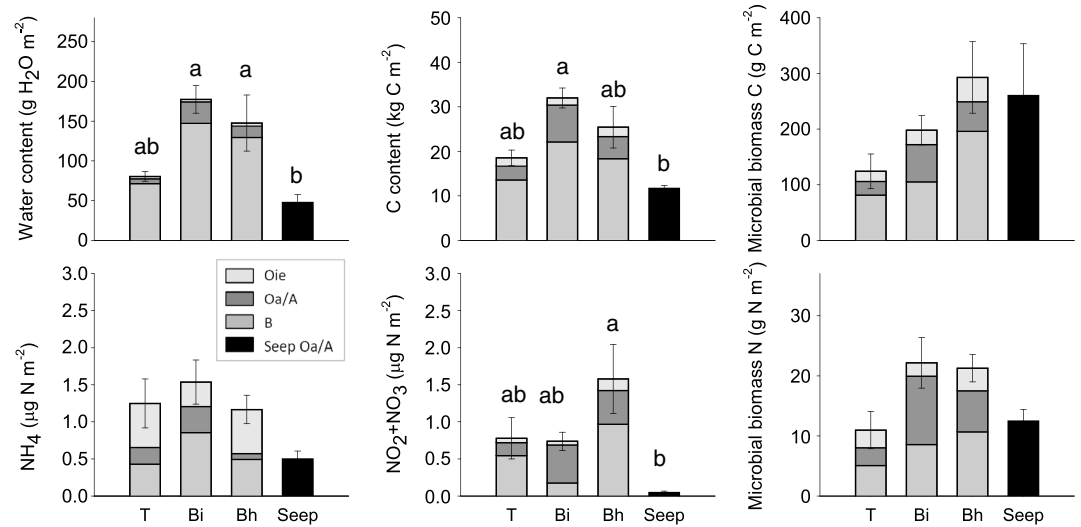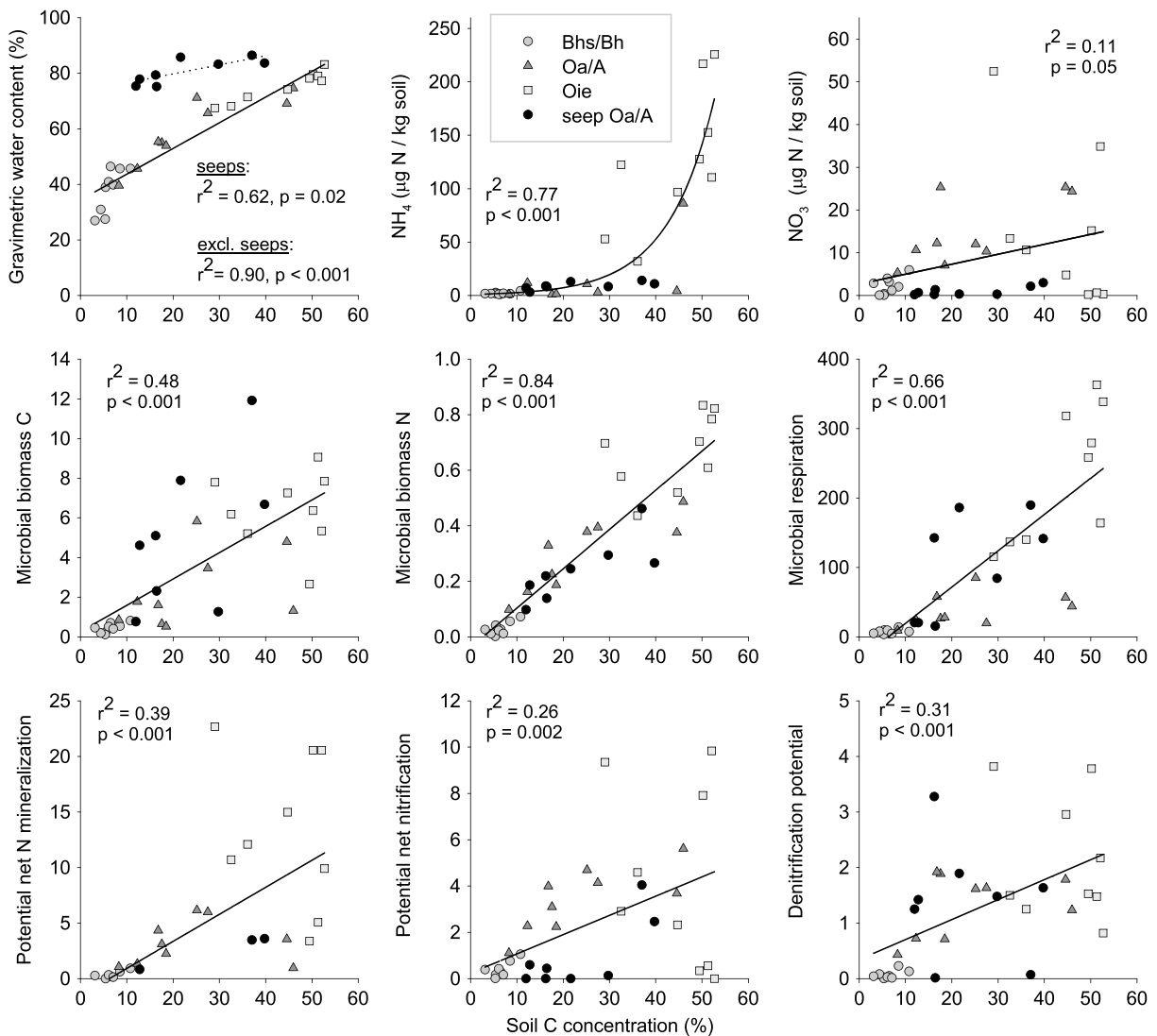
Figure 4. Soil biogeochemical variables (areal basis) by soil horizon and hydropedologic setting (T: typical podzol, Bi: bimodal podzol, and Bh: Bh podzol). Different letters indicate significant differences at $P < 0.10$.

potential microbial respiration ($r^2 = 0.66$), and microbial biomass C ($r^2 = 0.48$), with positive linear relationships across soil horizons and seeps. Soil NH_4^+ ($r^2 = 0.77$) had a strong exponential relationship with soil C concentration content across soil horizons and seeps (Figure 6). The remaining variables (potential net N mineralization ($r^2 = 0.39$), denitrification potential ($r^2 = 0.31$), potential net nitrification ($r^2 = 0.26$), and soil NO_3^- concentration ($r^2 = 0.11$)) had significant but weaker positive linear relationships with soil C concentrations across all the sampling locations (Figure 6).

Using GIS to estimate areal coverage by hydropedologic setting, we found that typical podzols dominate Watershed 3 (25 ha), followed by Bh podzols (4.6 ha), with E podzols, Bhs podzols, and bedrock each accounting for 3.9 ha. Bimodal podzols (1 ha) and groundwater seeps (0.1 ha) are minor components of the

Figure 5. Soil C and N cycling variables (areal basis) by soil horizon and hydropedologic setting (T: typical podzol, Bi: bimodal podzol, and Bh: Bh podzol). Different letters indicate significant differences at $P < 0.10$.

Figure 6. Soil biogeochemical variables and processes (mass basis) as a function of soil C concentration, by soil horizon. Regressions are drawn with solid lines to indicate the best fit (linear except in A and B) across soil horizons and seeps, except in A, where seeps are indicated with a separate linear regression and a dotted line, while a nonlinear relationship for other horizons is shown with a solid line.

watershed on an areal basis. Using mean rates and profile thicknesses for typical podzols only, we found that profile estimates increased by 5 to 15% (±1–2%) when the deeper parts of B horizons were included in contrast to the standard approach (Table 2). When rates, profile characteristics, and estimated areas specific to each hydropedologic setting were used to calculate the watershed level rates, we found that rates were 21–27% (±17–19%) higher than those estimated using the complete profile of typical podzols and 34–43% (±17–19%) higher than those rates calculated conventionally using only the top 10 cm of mineral soil, with increases in microbial respiration by 43% (24–61%), potential net N mineralization by 42% (24–61%), potential net nitrification by 39% (20–58%), and denitrification potential by 34% (17–52%).

4. Discussion

4.1. Do Hydropedologic Settings Have Distinctive Biogeochemistry?

The primary objective of this research effort was to determine whether morphologically distinct hydropedologic settings had distinct biogeochemical signatures, in terms of C and N cycling in the landscape. We analyzed soils for multiple variables related to C and N biogeochemistry, examining differences between

Table 2. Areal and Watershed-Wide Estimates of Potential C and N Cycling Rates in Watershed 3, Hubbard Brook Experimental Forest[a]

	WS3 2010				
Areal Means	**10 cm B Horizon**	**Full Profile**	**Percent Change**[b]		
Potential net N mineralization (mg N m^{-2} d^{-1})	125 ± 2.8	142 ± 2.8	14 ± 2		
Potential net nitrification (mg N m^{-2} d^{-1})	140 ± 2.0	161 ± 2.0	15 ± 1		
Denitrification potential (mg N m^{-2} h^{-1})	40.0 ± 0.6	42.1 ± 0.60	5 ± 2		
Potential microbial respiration (mg C m^{-2} d^{-1})	2.16 ± 0.03	2.44 ± 0.03	13 ± 2		
Watershed Estimates (42.4 ha)	**10 cm B Horizon**	**Full Profile**	**Profile by HPS**	**Percent Change**[c]	**Percent Change**[d]
Potential net N mineralization (kg N/d)	48.2 ± 1.1	54.8 ± 1.1	69 ± 9	25 ± 19	42 ± 19
Potential net nitrification (kg N/d)	54.2 ± 0.8	75.4 ± 0.8	75 ± 10	21 ± 19	39 ± 19
Denitrification potential (kg N/h)	15.4 ± 0.2	16.2 ± 0.2	21 ± 3	27 ± 17	34 ± 17
Potential microbial respiration (Mg C/d)	0.83 ± 0.01	0.94 ± 0.01	1.2 ± 0.1	27 ± 18	43 ± 18

[a]Values were calculated from the mean rates and profile thickness (10 cm of B horizon as well as full profile) for typical podzols, and from hydropedologic setting (HPS)-specific rates, profile thicknesses, and estimated areas.
[b]Percent change in estimates when including the more complete typical podzol profile we described versus the conventional method including only the top 10 cm of mineral soil.
[c]Percent change in estimates when including rates, profile thicknesses, and areas specific to each hydropedologic setting versus the conventional method including the top 10 cm of mineral soil.
[d]Percent change in estimates when including rates, profile thicknesses, and areas specific to each hydropedologic setting versus the more complete typical podzol profile.

hydropedologic settings and soil horizons on a mass basis, then on an areal basis by adjusting for the bulk density and thickness of each horizon in each soil pit. For all the variables we examined, our data did not support our expectations that C and N pools and process rates would be different across hydropedologic setting; rather, more dramatic differences were observed across soil horizons.

The B horizons had generally smaller C and inorganic N pools and slower rates of biogeochemical activity on a mass basis compared to other horizons (Table 1 and Figure 3), as has been found in other studies [*Jobbágy and Jackson*, 2000; *Johnson et al.*, 2000; *Sanderman and Amundson*, 2010]. Because the hydropedologic settings vary in terms of horizon thickness as well as morphology, our goal was to determine whether the full soil profiles might be different by hydropedologic setting, as the contribution of B horizons would be especially variable due to differing thickness among hydropedologic settings. The spodic hydropedologic settings and seeps were most markedly different, with smaller inorganic N and organic C pools and lower net N mineralization and net nitrification potentials in seeps. We found few differences among the spodic hydropedologic settings. While similarities in C quantity and quality in the subsurface horizons seem to control microbial C and N cycling processes, resulting in low observed variability, B horizons in this watershed feature distinct biogeochemical signatures (e.g., as seen in the accumulation of aluminum and iron oxides in Bhs horizons, finer structure in Bh horizons due to deposition of spodic material, and differing saturation frequencies) that are likely to affect hydrology, redox conditions, and metal chemistry [*Bailey et al.*, 2014].

Findings from our study did not support our hypothesis that Bh podzols, as zones of accumulation along hydrologic flowpaths, would represent biogeochemical hot spots in the landscape, perhaps due to insufficient sample size. The interaction of horizon-specific biogeochemical activity and thickness specific to each hydropedologic setting did produce interesting patterns for most variables. The forest floor (Oi/Oe) and Oa/A horizons, which varied little in thickness across hydropedologic settings, were the dominant zones for some N-related variables, accounting for the largest portion of potential net N mineralization (71% ± 18%) and potential denitrification (73% ± 21%). In contrast, B horizon thickness and activity accounted for more of the total soil profile water content (86% ± 14%) and soil organic C (71% ± 11%). We found that the surface horizons and mineral horizons contained similar pools of inorganic N and microbial biomass C and N and comparable rates of soil microbial respiration and potential net nitrification. *Bohlen et al.* [2001] also showed the importance of mineral soil horizons to C and N pools and biogeochemical activity, with the top 10 cm of mineral soils accounting for between 24% (NH$_4^+$ availability) and 52% (total inorganic N) of measured variables in surface soils. More detailed studies, with high-resolution sampling of multiple variables are ongoing in Watershed 3, and biogeochemical differences between hydropedologic settings may emerge from these studies.

Despite having been sampled only to 15 cm depth (versus up to 76 cm in Bh podzols), groundwater seeps were found to have similar areal rates of microbial respiration and denitrification potential. In these zones,

NO_3^- pools, net nitrification, and net N mineralization rates were relatively low, suggesting that C availability and soil moisture were high enough to promote denitrification, resulting in low rates of net N mineralization and net nitrification. This evidence supports the idea that groundwater seeps may be hot spots of denitrification in the landscape and that denitrification can be so dominant that it depletes pools of inorganic N [*Lohse et al.*, 2013]. These seeps, along with riparian zones that are known to be important for denitrification in watersheds [*Ashby et al.*, 1998; *Vidon et al.*, 2010], are likely to remove NO_3^- inputs from atmospheric deposition and from groundwater sources that converge where conditions for denitrification are favorable, resulting in regulation of NO_3^- that could be significant at the watershed scale [*Dittman et al.*, 2007; *O'Driscoll and DeWalle*, 2010; *Zimmer et al.*, 2013].

These results have implications for watershed-scale biogeochemical assessments in the northern hardwood forest ecosystems and landscapes at the HBEF. While it is possible to distinguish and map distinct spodic hydropedologic settings within these landscapes, the lack of striking differences in microbial C and N cycle processes across the spodic profiles that we sampled (which exclude E and Bhs podzols in the catchment) suggests that the spodic components of the landscape have relatively homogeneous patterns of biogeochemical processes. While hydrologic processes and landform create observable hydropedologic variability within the landscape, the lack of variation in organic matter quality (vegetation varies little with hydropedologic settings investigated in this study) and soil moisture at the time of sampling leads to relatively homogenous patterns of biogeochemical activity within the spodic hydropedologic settings at the height of the growing season. There were marked differences between the spodic hydropedologic settings and the seeps, but these have different vegetation and saturated soil conditions that lead to high levels of soil moisture, organic matter (quantity and quality), and biogeochemical processes. Thus, the hydropedologic approach was useful for highlighting which mappable differences are functionally significant and which are not.

4.2. The Importance of Deeper Horizons

The C and inorganic N contents and biogeochemical activities in the B horizons were substantial, similar to the findings of large C and N pools in mineral horizons in other watersheds at HBEF [*Johnson et al.*, 2000]. Because we collected grab samples within the sampled soil horizons instead of quantitatively measuring C stocks in the soil pits, we may have overestimated deep soil C pools [*Rau et al.*, 2011; *Vadeboncoeur et al.*, 2012]. Our measurements from WS3 fall within the range of values in the long-term record of microbial biomass and activity measured in reference plots at the HBEF each July from 1996 to 2012 (http://hubbardbrook.org/data/dataset.php?id=67). Based on the sampling approach used for the long-term record (Oi/Oe + Oa/A + 10 cm of mineral soil), we compared our values for the dominant soil type (typical podzols) in WS3 based on these depth increments (Table 2). When the full profile was considered, including results for B horizons below 10 cm, the WS3 estimates increased modestly, by 5 to 15% (±1–2%). However, once the interacting effects of biogeochemical process rates, horizon thicknesses, and areal coverage specific to each hydropedologic setting were incorporated, estimated rates were 34–43% (±17–19%) higher than conventionally upscaled results for typical podzols. These results confirm that lower soil horizons and hydropedologic settings play an important role in the biogeochemistry of the forest ecosystems at the HBEF. This importance is likely enhanced at the HBEF, where soils are shallow and significant amounts of water, carbon, and nutrients are transported by shallow, lateral groundwater flow [*Detty and McGuire*, 2010; *Dittman et al.*, 2007]. Our results are also consistent with those reported by *Werner et al.* [2011], who reported low O_2 and significant levels of CH_4 and N_2O in shallow groundwater interacting with B horizons at our sites. Deeper soils, including these B horizons, thus can support a significant biogeochemical activity, including anaerobic processes [*Bailey et al.*, 2014], as has also been found by other researchers [*Groffman et al.*, 1996; *Gurwick et al.*, 2008; *Jobbágy and Jackson*, 2000; *Sanderman and Amundson*, 2010].

4.3. Biogeochemical Processes at the Landscape Scale

The values of all the measured variables increased significantly with increasing soil C concentration across all the horizons and spodic hydropedologic settings, with seeps fitting the general pattern for all the variables except gravimetric water content ($P < 0.05$; Figure 6). While NH_4^+ concentrations increased exponentially with increasing %C, all other variables showed linear relationships across the range of %C in seeps and spodic hydropedologic settings. These results show that soil microbial C and N cycling is tightly linked to soil %C

[*Booth et al.*, 2005], either directly by providing substrates for C and N uptake and subsequent transformation or indirectly by controlling water availability during the warmest months of the year.

It is important to note that microbial C and N cycling and soil inorganic N pools are more temporally dynamic than soil C concentrations. However, the strong link we found between water availability and soil %C suggests that during the growing season, microbial processes throughout soil profiles and across watersheds are mappable based on soil C, as has been shown in other forests [*Cosandey et al.*, 2003; *Webster et al.*, 2011]. Although the relationships between soil microbial processes and soil C concentrations are likely to change seasonally based on forest phenology or in response to climate patterns, the potential microbial biomass and activity parameters that we measured show much less temporal variation than soil moisture and temperature [*Groffman and Tiedje*, 1989], which supports the idea that the relationships reported here are not ephemeral. Furthermore, the patterns observed in deeper soils are likely to be even more temporally stable due to the lower amplitude of temperature variation at depth. Thus, the hydropedologic approach, where morphologically distinct settings in the landscape that feature contrasting and predictable soil C contents can be identified, may be useful for estimating and mapping microbial C and N cycling rates at the watershed scale. Other work in Watershed 3 is exploring how hydrologic variability and flowpaths at finer temporal and spatial scales relate to other biogeochemical processes and has helped identify likely biogeochemical hot spots [*Bailey et al.*, 2014].

5. Conclusions

While variation in morphology and profile development in spodic hydropedologic settings did not translate into significant differences in biogeochemical C and N cycling in this study, the contrasts between spodic hydropedologic settings and seeps were more striking, with high rates of microbial respiration and denitrification potential in seeps on an areal basis. We found that including rates, horizon thicknesses, and watershed coverage specific to each hydropedologic setting produced estimates of C and N cycling rates at the watershed scale that were 23–28% higher than conventional upscaling methods. The hydropedologic approach provided insights that helped to recognize the importance of deeper mineral soil horizons that have low C concentrations relative to surface horizons, understand the processes that lead to their formation in the landscape, and develop a framework for predicting the location of morphologically distinct hydropedologic settings in the landscape. Given the strong links between soil %C and C and N cycling across all the soil horizons and hydropedologic settings, along with distinct soil profile development in contrasting hydropedologic settings, this approach may be of great utility in more accurately estimating and modeling C and N biogeochemical processes at the watershed scale.

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