Title: Land-Use and Land-Cover Effects on Soil Microbial Community Abundance and Composition in Headwater Riparian Wetlands

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Abstract

Headwater riparian wetlands are relatively small in size but functionally significant as expected “hot spots” of microbial activity in the landscape. Despite their roles as biogeochemical drivers, little is known about how microbial communities in headwater riparian wetlands are affected by surrounding land-uses and land-covers (LULCs). The primary objective of this study was to determine if and how wetland soil microbial abundance and community composition varied as a function of landscape metrics as mediated through on-site edaphic properties. Forty-two soil samples, collected from eight headwater riparian wetlands in the Ridge and Valley Region of central Pennsylvania, were used for phospholipid fatty acid (PLFA) profiling of soil microbial communities. These samples were used to create microbial habitat models describing plot-level relationships between edaphic properties and microbial measures (i.e., microbial biomarker abundances, ratios and composition). Soil organic matter (SOM) content was a strong predictor of microbial biomarker abundances and fungi / bacteria ratios, while soil pH was a strong predictor of microbial composition (i.e., relative abundance of individual fatty acids) and potential microbial stress indices (i.e., $\text{cy19:0a}/\text{18:1}\omega7\text{c}$ and $\text{cy17:0}/\text{16:1}\omega7\text{c}$). Soil texture, soil moisture, and litter total nitrogen had smaller, but significant effects in these empirical microbial habitat models. Microbial habitat models were subsequently used to estimate microbial measures for a larger regional headwater riparian wetland dataset ($n = 87$), where edaphic property information was compiled. Site-average microbial measures were correlated with wetland elevation, and with landscape composition metrics in a landscape assessment area (i.e., $125,664 \text{ m}^2$). Wetland elevation explained high among-site variability in microbial abundance measures, as mediated through SOM content, in headwater riparian wetlands in forested landscapes. However, wetland elevation was confounded by landscape composition, for

46 headwater riparian wetland in mixed forested-agricultural landscapes. Hydrology,
47 geomorphology, and changes in vegetation could be used to explain SOM variation across both
48 elevation and landscape composition. Microbial composition measures, including the
49 cy19:0/18:1 ω 7c ratio and principal component (PC) axes derived from 23 individual PLFA
50 biomarkers were negatively related to the Landscape Development Index (LDI) metric, and the
51 percent area of row crops, pastures and grasslands, and positively related to percent area forest.
52 The cy17:0/16:1 ω 7c ratio was positively related to the LDI metric, and percent area of pasture
53 and grasslands, and negatively related to percent area forest and wetland elevation. Differences
54 along PC axes were most clearly related to the relative abundances of fungal communities;
55 headwater riparian wetland in forested landscapes had significantly higher relative abundances of
56 saprophytic fungi biomarkers, while headwater riparian wetland in mixed landscapes had
57 significantly higher relative abundances of the arbuscular mycorrhizal fungi biomarker. This
58 study highlights the utility of simple landscape metrics, which describe wetland position and
59 landscape composition, for predicting differences in edaphic variability and associated microbial
60 community composition and biomass among headwater riparian wetlands at a regional scale.

61 **1. Introduction**

62 The importance of characterizing wetland functioning for the delivery of services such as
63 carbon sequestration and nitrogen removal is at the forefront of research and management
64 efforts, exemplified by the first ever National Wetland Condition Assessment performed in the
65 United States (U.S.) by the Environmental Protection Agency (EPA) in 2011. With
66 advancements in molecular tools, the use of microbial indicators in wetland condition
67 assessments has been advocated by Sims et al. (2013). Some wetland soil processes (e.g.,
68 nitrification, methanogenesis, and sulfur reduction) are directly affected by microbial composition
69 due to the specificity of the microbial groups required to perform these functions (Schimel and
70 Gullledge, 1998). However, more general processes, such as decomposition, can also be affected
71 by microbial composition, particularly in the rhizosphere, in above ground litter, and in mineral
72 soils over temporal scales where microbial resource allocation drives system dynamics (Schimel
73 and Schaeffer, 2012). For example, fungi are expected to metabolize complex substrates more
74 directly than bacteria (Holland and Coleman, 1987). Fungal biomass is also more resistant to
75 decomposition than bacterial residues (Guggenberger et al., 1999), with an increase in fungal
76 biomass expected to favor the accrual of soil carbon through the formation of stable soil structure
77 (Allison et al., 2005; Jastrow and Miller, 1998). Further comparisons have revealed that
78 decomposition and carbon accretion rates in soil can vary among fungal groups (Averill et al.,
79 2014; Hodge et al., 2001).

80 While several studies have assessed differences in wetland microbial communities or
81 specific functional microbial groups in created wetlands and wetland restoration efforts (Card
82 and Quideau, 2010; Hartman et al., 2008; Peralta et al., 2010; Sims et al., 2012; Smith et al.,
83 2007), we lack knowledge about differences in wetland microbial communities across variability

in wetland landscape contexts. In this study we begin to fill this gap by assessing variability in microbial communities across various dimensions of landscape context. Landscape context can be described by factors such as landscape position (e.g., elevation) and changes in the composition and/or arrangement of land-use and land-cover (LULC) types in the areas surrounding wetlands. While being spatially separated, the surrounding landscape can be energetically linked to wetlands, through lateral flow (i.e., surface and subsurface) of water, sediments, and chemical constituents. We hypothesize that these linkages can play a major role in microbial community composition and activity by affecting edaphic properties and hence the functions which wetlands provide. We suggest this is particularly true for headwater riparian wetlands, which are commonly situated downslope of upland areas in the watershed and receive surface, subsurface, and overbank flow. These systems are considered “hot spots” in the landscape for biogeochemical activities (McClain et al., 2003; Vidon et al., 2010), and lie adjacent to headwater streams, which constitute 60 to 75 % of total stream and river miles in the U.S. (Leopold et al., 1964). Their small size relative to the surrounding watersheds, and their high perimeter to area ratios also make them potential “hot spots” of surrounding landscape influence.

Although linkages between wetland microbial communities and landscape context, particularly landscape composition, are not well understood, shifts in other on-site properties have been well documented in headwater riparian wetlands and related to changes in landscape composition. For example, in the Ridge and Valley region of central Pennsylvania headwater riparian wetlands with surrounding landscapes dominated by agricultural and urban LULC categories have greater percentages of herbaceous cover and a predominance of invasive and generalist species, compared to wetlands in landscapes dominated by forest (Miller and Wardrop,

2006; Moon and Wardrop, 2013). Hydrologic disconnections between streams and adjacent wetlands have also occurred (Adamus et al., 2001; Ryan, 2005), leading to changes in the frequency and intensity of inundation (Moon and Wardrop, 2013; Wardrop et al., In Press) and the delivery of sediment. ‘Reference standard’ wetlands (i.e., those deemed to be in the most pristine condition) tend to have lower sedimentation rates (Wardrop and Brooks, 1998), higher levels of soil organic matter (SOM) (Cleveland et al., 2011; Cohen et al., 2005; Dinesh et al., 2004; Freeland et al., 1999; Moon and Wardrop, 2013; Reiss, 2006; Rokosch et al., 2009; Spencer et al., 1998) lower soil bulk densities (Innis et al., 2000; Pennings et al., 2002; Reiss, 2006; Spencer et al., 1998), and lower soil pH (Cleveland et al., 2011; Moon and Wardrop, 2013; Reiss, 2006) compared to those which are considered ‘impacted’ or ‘degraded’.

Changes in these wetland on-site properties further support hypothesized changes in the microbial communities because many of these on-site factors act as proximal controls of microbial communities, and thus mediate impacts arising from landscape context (distal controls). Some studies have successfully linked microbial composition and biomass directly to ecosystem type, land-use change, management regimes, and stressors within ecosystems (Allison et al., 2005; Burton et al., 2010; Drenovsky et al., 2010; Xue et al., 2008). Differences in soil microbial biomass and composition have been associated with changes across hydrologic gradients, flooding duration, water table depth and variability, and rates of sedimentation (Balasooriya et al., 2008; Lockaby et al., 2005; Mentzer et al., 2006; Unger et al., 2009; Yu and Ehrenfeld, 2010). Others have shown that soil microbial communities are strongly influenced by vegetation (Wardle et al., 2004), with a number of studies linking microbial community composition, diversity, and enzyme activity to specific plant species (Borga et al., 1994; Ehrenfeld et al., 2001; Ingham and Wilson, 1999; Kourtev et al., 2003, 2002; Sundh et al., 1997)

and higher levels of ecological complexity, such as aboveground plant diversity (Carney and Matson, 2006). Further, links have been made more directly between microbial measures and differences in edaphic characteristics (Balasooriya et al., 2008; Calderón et al., 2001; Fierer and Jackson, 2006; Frostegård et al., 1997; Gutknecht et al., 2006; Lauber et al., 2008; Wakelin et al., 2008; Yu and Ehrenfeld, 2010). Litter total carbon:total nitrogen ($L_{TC:TN}$) ratios (Carney and Matson, 2006; Myrold, 1999), soil temperature (Gray et al., 2011; Klamer et al., 2000; Pietikäinen et al., 2005), soil moisture (Bossio and Scow, 1998; Langer and Rinklebe, 2009), carbon and nutrient availability (Fierer et al., 2007; Högberg et al., 2007), soil pH (Fierer and Jackson, 2006; Högberg et al., 2007), and soil texture (Gregorich et al., 1991; Ludvigsen et al., 1999) have all been shown to influence microbial abundance, composition, and in some cases microbial diversity (Fierer et al., 2007).

We aimed to identify links between distal controls in the surrounding landscape and microbial communities in headwater riparian wetlands, as they are mediated through proximal links to edaphic (i.e., surface litter and soil) on-site conditions. In this study we used landscape metrics that described landscape position (i.e., elevation), surrounding landscape composition (i.e., land cover class percentages) and levels of anthropogenic use (i.e., landscape development intensity index). In a select set of headwater riparian wetlands ($n = 8$) located in the Ridge and Valley region of central Pennsylvania we asked, (1) at the plot-level, what edaphic properties best explain the range of variability found in microbial communities (i.e., microbial biomarker abundances, ratios and composition) across headwater riparian wetlands? We used phospholipid fatty acid analysis (PLFA) to characterized microbial communities, and developed microbial habitat models by relating microbial measures to edaphic conditions. We then asked, (2) do microbial communities differ with landscape metrics? To address this question we used two

analyses. First, we compared microbial measures from plots at the eight headwater riparian wetlands where PLFA data were collected. Four of these study sites were located in natural landscapes and four were located in mixed LULC landscapes. In this region, natural landscapes are described by high forest cover, while mixed landscapes are primarily a combination of forest and agricultural (i.e., row crop, grassland and pasture) LULC classes. Second, we compiled a soil property dataset from 79 additional headwater riparian wetlands in the region and used the microbial habitat models to predict changes in microbial measures across these sites. Both datasets were combined to look for relationships between microbial measures and landscape metrics after controlling for any spatial autocorrelation at the regional scale.

2. Methods

2.1 Site and Plot Selection

Previous studies (Auman, 2004; Bishel-Machung et al., 1996; Bishel, 1994; Cleveland et al., 2011; Drohan and Brooks, 2013; Williams-Tober, 2009; Riparia, unpublished) were used to compile edaphic property information for headwater riparian wetlands across the Ridge and Valley region of central Pennsylvania (herein, the regional dataset sites). A total of 87 headwater riparian wetlands were identified across these studies (**Figure 1**), and are part of The Pennsylvania State University Riparia reference wetland collection database (i.e., http://wetlands.psu.edu/projects/reference_wetlands.asp, last accessed 9/12/2015). At these 87 headwater riparian wetlands, surface soil samples (i.e., 5 cm depth) were collected and analyzed between 1993 and 2006. The total wetland assessment area (900 – 10,000 m²) and the number of samples collected within each wetland assessment area (n = 1– 16) ranged across the studies. Some wetland assessment areas were sampled across multiple studies, but in different years.

Eight of these headwater riparian wetlands (i.e., herein, PLFA profiling sites), representing sites in natural (i.e., predominately forested) and mixed (i.e., predominately forested and agricultural areas) landscapes, were selected for additional sampling in August of 2007 (**Table 1**). These selected sites were used to construct empirical microbial habitat models (i.e., microbial – edaphic property relationships), which could be applied to the larger regional dataset to predict microbial measures. Plots (1-m²) used in the construction of empirical microbial habitat models were chosen to represent the range of known soil conditions (e.g., SOM, pH, soil moisture) found within and across the eight selected headwater riparian wetlands. To select these plots we used a larger set of 1-m² edaphic sampling plots (n = 337) that were distributed across the eight sites (**Figure 1**) (Moon et al. 2012); each headwater riparian wetland assessment area (2,025 m²) had plots (30 to 45 plots per site) arranged along three transects perpendicular to their respective mainstream channels (**Figure 2**). Ward’s minimum variance method of hierarchical clustering was used with plot soil property information to identify the possible range of soil habitats in JMP® (Version 9, SAS Institute, Inc.). The number of clusters (n = 7) was determined through inspection of the dendrogram scree plot. Sites varied in the number of edaphic habitat clusters that they were included in; thus the number of PLFA profiling plots varied across sites (**Table 1**). Two plots were selected for PLFA profiling from each site within each cluster group as long as at least three plots at the site were within that cluster. This led to a total of 42 plots for use in PLFA profiling. The edaphic sampling plots that were not selected for PLFA profiling, are not included in the analyses, described below.

2.2. Sample Collection

In each 1-m² plot a 100-point grid (i.e., resolution = 10 cm x 10 cm) was used along with a random number generator to establish 16 soil-sampling points; the spatial pattern of the

sampling points varied across all plots. At four of the 16 sampling points all surface litter (i.e., dead leaves and small twigs) was collected from 81-cm² circular areas (i.e., diameter ~ 10.2 cm) and pooled into a composite sample. At the other 12 sampling points, surface litter was removed prior to soil sampling. Once surface litter was removed, volumetric water content (Θ_v) was measured to a depth of 5 cm and averaged across all 16 sampling points (EC-5 soil moisture probe, Decagon Devices).

Soil samples were then collected at each of the 16 sampling points (sampling point core area = 2.27 cm²). Five cm of mineral horizon soil was collected at each of the sample points and composited to represent each plot. However, at three of the eight sites, some plots had sampling points with an organic horizon present above the mineral horizon. In plots where this occurred, we recorded the depth of the organic horizon at each sampling point and composited the entire organic layer into a separate plot sample. Both organic and mineral soil samples were used in the cluster analysis described above and therefore both were subsequently considered for PLFA profiling. However, to maintain independence of sampling units, PLFA profiling was not performed on both the composited organic horizon sample and the composited mineral horizon sample that came from the same plot. Rather, each of the 42 plots represented either a mineral horizon sample (n = 31) or an organic horizon sample (n = 11) that best characterized the surface soil layer; mineral horizon samples came from plots where the organic layer was shallow (i.e., average plot organic horizon depth between 0 and 1.3 cm), while organic horizon samples came from plots that had thicker organic horizon layers (i.e., average plot organic horizon depth between 1.0 and 3.2 cm).

2.3 Edaphic Properties

Edaphic properties were measured at the 42 plots from the eight sites used for PLFA profiling. Surface litter was dried (Hotpack Dryers, Model # 21430) at 58 °C for approximately 48 hours or until mass change was < 1 %. Samples were weighed to calculate litter biomass ($Litter_{Biomass}$). Dried litter was ground using a Wiley Mini-Mill Model L10 (Thomas Scientific, Model # 3383-L10) to mesh size 40 (420- μ m mesh) and total carbon ($Litter_{TC}$) and total nitrogen ($Litter_{TN}$) were analyzed using combustion with a CE Instruments (Thermo Electron ©) Elemental Analyzer EA 1110 with a thermal conductivity detector.

Soil samples were frozen at -20 °C and subsequently freeze dried in a pilot lyophilizer (Virtis Company). Soil mass was taken before and after freeze-drying to determine gravimetric water content ($\Theta_g \pm 0.01$). Dried soils were passed through a 2-mm sieve to remove non-soil fractions. Soil pH was measured in a 2:1 ratio of deionized water to soil (Thomas, 1996). Soils were extracted using 2M KCl in a 1:10 ratio of soil to extractant (Keeney and Brenner, 1966), and nitrite-nitrate ($N-NO_2^- + NO_3^-$) and ammonium ($N-NH_4^+$) concentrations were colorimetrically determined using a Lachat QuikChem® 8500 Series 2 Flow Injection Analysis System (Lachat QuikChem® method 12-107-04-1-F and 12-107-06-3-B).

SOM content was measured using loss on ignition (LOI), following Nelson and Sommers (1996) with modification by Ben-Dor and Banin (1989). Subsamples were also sent to the Penn State Agricultural Analytical Service Laboratory for measures of Mehlich 3 phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) (Wolf and Beegle, 1995), and cation exchange capacity (CEC) (Ross and Ketterings, 1995). Finally, particle size analysis was conducted on mineral horizon soil fractions, including organic horizon sample plots (i.e., for organic horizon samples the underlying mineral soils were used for this analysis), to estimate soil texture properties. Organic matter was digested using 6 % hydrogen peroxide in an oven at 60

°C, and particle size was determined on 3 to 6 aliquots (depending on sample measurement variability) using a Laser Diffraction Particle Size Analyzer (Horiba, Model # LA-950 A2). Clay, silt and sand fractions were calculated following Wentworth's classification scheme (Wentworth, 1922).

2.4 PLFA Analysis

We used PLFA soil profiling in order to assess difference in microbial communities across on-site edaphic conditions and landscape metrics (described below). PLFAs are key structural components of the cell membrane of bacteria and macro-eukaryotes. They are thought to degrade rapidly with cell death (White, 1988; White et al., 1979), and as such PLFA abundance estimates have been successfully correlated with other measures of microbial biomass (Bailey et al., 2002; Leckie et al., 2004; Zelles and Bai, 1993), including those in floodplain soils (Rinklebe and Langer, 2010). Some PLFAs (Haack et al., 1994; O'leary and Wilkinson, 1988) have been used as biomarkers for specific functional groups of microbes (White et al., 1996) and with careful use can be a sensitive method for showing differences in microbial communities in soils (Frostegård et al., 2010).

A large number of fine roots remained in the soil samples after the 2-mm sieving. This was problematic for PLFA profile analyses because fungal biomarkers used in this study have also been identified as common to other eukaryotes (Frostegård et al., 2010). However, these markers have been shown to be good indicators of fungal relative abundances, particularly in forested soils (Frostegård et al., 2010; Kaiser et al., 2010), when other eukaryotes are removed from the samples (Zelles, 1999). Therefore all samples were run through an additional 0.5-mm sieve, where roots were handpicked from the sieve and soil particles were returned to the sample.

Adapted from Bligh and Dyer (1959), lipids were extracted from the soil using a single-phase mixture of chloroform, methanol, and phosphate buffer (pH 7.4) in a ratio of 1:2:0.8. Lipid classes were separated using silicic acid column chromatography (Zak et al., 1996) and neutral and phospholipids were methylated using a mild-alkaline solution (White et al., 1979). The solvent was evaporated under the N-EVAP (116 N Evap System, OA-SYS Heating System, Organomation Association) at 37 °C and stored in the freezer until analysis.

The fatty acid methyl esters (FAMES) were re-suspended in hexane and were analyzed using high-resolution fused-silica capillary gas chromatography (GC) on a HP 6890 with a 7683 autosampler (Agilent Technologies). A 25-m HP5-MS column (Agilent 19091S) was used with hydrogen as the carrier gas, and hexane as the solvent. A 1- μ L splitless injection was used to sample each vial. Fatty acids were identified by retention time in comparison with known standards and quantified using a FAME 19:0 internal standard (Matreya Inc.).

Microbial measures were calculated as molar abundances ($\text{nmol} \cdot \text{g}^{-1} \text{ soil}$) and fatty acid nomenclature, provided within, follows Tunlid and White (1992) (**Table 2**). Gram negative (gram⁻) bacteria and firmicutes were estimated by summing monosaturated fatty acids and terminally branched saturated fatty acids, respectively. The abundance of actinobacteria was estimated by summing mid-chain branched saturated fatty acids. Saprophytic fungi (SapF) were estimated by summing 18:1 ω 9c and 18:2 ω 6 fatty acids. PLFA 18:2 ω 6 has also been associated with ectomycorrhizal fungi (e.g., Kaiser et al. 2010); however, we found no significant relationship between 18:2 ω 6 and percent basal area of ectomycorrhizal trees at the plot-level (i.e., trees within a 10 m radius of 1-m² sampling plots) or at the site-level (i.e., trees within 10,000 m² assessment area) across our eight PLFA profiling sites (results not shown). PLFA 16:1 ω 5 is thought of as a potential biomarker for arbuscular mycorrhizal fungi (AMF) depending

on the ratio of the neutral lipid fatty acids (NLFAs) to the PLFAs. A ratio of < 1 is associated with bacteria, while a ratio ≥ 1 (1 to 200) is associated with AMF (Olsson, 1999). Total PLFA content was estimated by summing all bacteria (i.e., gram⁻, firmicutes, actinobacteria) and fungi (i.e., SapF and AMF, where NLFA / PLFA ≥ 1) PLFA biomarkers. Ratios including fungal to bacterial (F/B) fatty acids, and cyclopropyl (cy19:0a, cy17:0) to cis (16:1 ω 7c, 18:1 ω 7c) fatty acids were also calculated.

2.5 Landscape Metrics

Landscape metrics were calculated for the PLFA profiling sites and for the regional edaphic dataset sites. To capture distal controls of surrounding LULC, a landscape assessment area had to be defined. Brown and Vivas (2005) suggested the use of a 100-m buffer surrounding a wetland's edge as an assessment area of condition. However, many forested wetlands are not captured by land cover imagery (e.g., not included in the National Wetland Inventory, <http://www.fws.gov/wetlands/>, last accessed: 9/12/2015), are relatively narrow in shape, and run along continuous stretches of complex channel structures. Thus, the notion of a 100-m buffer around the edge of a wetland is not applicable for systems used in this study. Since most headwater riparian wetlands in the Ridge and Valley region are less than 1 ha (10,000 m²) in size, we selected a 125,664-m² (i.e., 200-m radius) circular area centered on the sampling site as a practical approach for characterizing the surrounding landscape. The extent of the wetland assessment area (i.e., the extent within which all sampling plots were established at a site) at PLFA profiling sites and regional edaphic dataset sites constituted between 0.7 – 7.9 % of the landscape assessment area (i.e., wetland assessment areas ranged between 30 – 100-m²).

Within each landscape assessment area, percent area cover was calculated for LULC categories using the 2005 PAMAP Digital Base Map (The Pennsylvania State University, 2007)

in Arc GIS (version 10.3, ESRI, Inc., © 1999-2014). Final categories included percent area of roads, row crops, pastures and grasslands, open water, NWI (i.e., National Wetland Inventory) emergent wetlands, forests (including: deciduous, coniferous, mixed and NWI forested wetlands), bare ground, residential areas, and commercial areas. The Landscape Development Intensity (LDI) index (dimensionless) was subsequently calculated for each landscape assessment area. The LDI was originally proposed by Brown and Vivas (2005), and estimates potential human impact in the landscape assessment area by summing weighted averages of percent area of LULC categories. Weights were cross-walked from Brown and Vivas (2005) to comparable LULC categories in our study region. These weights are based on the emergy required to maintain the LULC category (Brown and Vivas 2005). Finally, wetland elevation was extracted from 1:24,000 digital elevation models (DEMs, USGS National Elevation Data Set). **Appendix A. Supplementary Data: Table S1** contains percent area cover metrics, the LDI index and elevations for all headwater riparian wetland used in this study. DEM data source information, and the crosswalk from Brown and Vivas (2005) for LULC category weights is also provided.

2.6 Statistical Analyses

First, we established proximal relationships between edaphic properties and microbial measures at the PLFA profiling sites. This allowed us to create plot-level empirical microbial habitat models. Stepwise regression (forward and backward), using the stepAIC() function in the MASS package (Venables and Ripley, 2002) of R (R Core Team, 2015) was used to find the best candidate models for predicting microbial measures (i.e., PLFA biomarker abundances and ratios) across edaphic properties for the PLFA profiling plots, including all plots (n = 42) and mineral plots alone (n = 31). We chose to perform this analysis with both sets of data because

we wanted to determine if patterns held after organic horizon samples were removed. We found that general patterns (i.e., predictors or highly correlated predictors, strength of relationships) were consistent across models using the full dataset and the dataset with mineral plots alone. Thus, models including all plots will be discussed within, while mineral plot models are provided in **Appendix A. Supplemental Table S2.**

Initial predictors included plot mean organic horizon depth, SOM content, soil moisture (Θ_g or Θ_v), pH, clay, silt and sand content, Litter_{Biomass}, Litter_{TC}, Litter_{TN}, and Litter_{TC:T_N} ratios. All of the predictors selected for use in empirical modeling were thought to be relatively stable across an annual cycle; this was previously confirmed (i.e., 3 to 4 sampling dates) for Θ_g , Θ_v , pH and Litter_{Biomass} using the larger database of plots ($n = 373$ plots) across the eight PLFA profiling sites (Moon, 2012). During modeling, predictors were compared using correlation coefficients and were further evaluated for removal due to multicollinearity if correlations exceeded 0.7 (i.e., Variance Inflation Factor ~ 1.96). We used Akaike information criterion (AIC), which provides a relative measure to assess the tradeoff between goodness of fit and model complexity, along with analysis of variance (ANOVA) results to resolve any final ambiguities about the removal of predictors. In a few cases non-linear functions were used to improve model fits.

In addition to PLFA ratios, microbial community composition was evaluated using principal components analysis (PCA) on the relative abundance of the individual ($n = 23$) PLFAs (% mole fraction) in the base package of R (R Core Team, 2015). A large proportion of the PLFAs used in this analysis exhibited linear behavior, and as such, PCA was deemed an appropriate tool for exploring differences among PLFA relative abundances (McCune and Grace, 2002). The first two PC axes of the ordination were regressed against edaphic properties to

create empirical microbial habitat models, following regression procedures described above. Similar to abundance analyses, these analyses were conducted for all PLFA profiling plots and mineral PLFA profiling plots alone; again, general patterns held. The ordination and PC axis models from all plots are discussed within, while the mineral plot ordination and PC axis models can be found in **Appendix A. Supplemental Figure S1 and Table S2.**

We then used two methods to assess relationships between microbial measures and the distal controls of landscape metrics. The first was a plot-level analysis performed on PLFA profiling sites alone; since a clear pattern emerged during sampling, where organic horizon soils were only present at some of the headwater riparian wetlands in forested landscapes (i.e., 3 of 4 study sites), we wanted to distinguish which differences in microbial measures could be attributed to this soil horizon effect vs. landscape context differences. We used two-way ANOVA tests in the base package of R (R Core Team, 2015) to compare edaphic properties and microbial measures (i.e., biomarker abundances, relative abundances, and ratios) between landscape contexts and soil horizons for the PLFA profiling plots. We split PLFA profiling plots between two landscape contexts: “forested” (i.e., $n = 4$, landscape assessment area with 90 – 100 % forest cover) and “mixed” (i.e., $n = 4$, landscape assessment area with 24 – 54 % forest cover). Forested landscape sites were further split between organic horizon plot samples and mineral horizon plot samples, while mixed landscape sites only had mineral horizon plot samples. This resulted in three landscape context - soil horizon subgroups, denoted in subsequent sections as: Forested-Organic (F-O), Forested-Mineral (F-M), and Mixed-Mineral (M-M) soils. We compared the results from the two-way ANOVAs to mixed effect models with a random effect of headwater riparian wetland study site, using the lme4 package (Bates and Maechler, 2010) in

R (R Core Team, 2015). However, including a random effect of site did not change significance differences between or among groups (results not shown).

In the second analysis we used both PLFA profile sites and regional edaphic dataset sites so that we could assess variability in microbial measures across a wider range of landscape contexts within the region. In order to include the regional edaphic dataset in this analysis we used the microbial habitat models to predict microbial abundance and composition measures for all plots within this dataset. Due to a lack of Litter_{TN} content data, F/B ratios could not be predicted for the regional edaphic dataset sites. To evaluate the extrapolative ability of the empirical microbial habitat models, we qualitatively compared the ranges of edaphic properties for plots from the PLFA profiling sites to plots from the regional edaphic dataset sites, when comparative datasets were available. Metadata on the edaphic property information synthesized from the regional data sources, including site classifications (i.e., NWI and hydrogeomorphic classifications), soil property sampling information, and references are provided in **Appendix A. Supplementary Data: Table S3.**

We used Mantel's tests to determine if headwater riparian wetlands with similar landscape metrics were also more similar in their microbial measures. To explore site-level relationships we averaged plot-level data for microbial measures, including field measurements at the PLFA profiling sites and predicted measurements at the regional edaphic dataset sites. Using simple Mantel's tests between geographic distance (predictor variable) and microbial measures (response variable), we were able to test for and subsequently control for (i.e., partial Mantel's tests) spatial autocorrelation in microbial measures and landscape metrics across the region. Mantel's tests allowed us to look for relationships between multivariate measures in addition to relationships between univariate measures (e.g., gram⁻ bacteria and percent area

forest cover). Multivariate dissimilarity matrices included the abundances (herein, community biomarker abundance measure) and relative abundances (herein, community biomarker relative abundance measure) of biomarker groups (i.e., gram-, firmicutes, actinobacteria, SapF, and AMF) for each site, and the percent area of LULC classes (i.e., roads, row crops, pastures and grasslands, open water, NWI emergent wetlands, forests, bare ground, residential areas, and commercial areas) for the landscape assessment area (herein, landscape percent area composition metric) around each site. Euclidean distance was used to construct all dissimilarity matrices and due to the violation of unequal variances, Mantel's tests were performed using non-parametric Spearman ranks. Tests were performed using the 'ecodist' package (Goslee and Urban, 2007) in R (R Core Team, 2015).

3. Results

3.1 Microbial Habitat Models

PLFA abundances ranged from 24 to 730 nmol·g⁻¹ soil across the 42 sampling plots at profiling sites. Strong (i.e., adjusted $R^2 > 0.70$) model fits were found between PLFA biomarker abundances and edaphic properties (**Table 3**). SOM content was the dominant predictor of abundance in all cases, with a positive effect. Small but significant improvements were observed in bacterial models and the AMF model by including soil texture and pH information, respectively. Clay content had a positive effect and sand content had a negative effect on bacterial biomarker abundances. pH had a positive effect on the abundance of AMF. Ratio models achieved moderate (i.e., adjusted $R^2 > 0.60$) to strong fits. SOM content was the dominant predictor in the F/B ratio model, with a secondary effect of Litter_{TN} content. Both SOM and Litter_{TN} content had positive effects on the F/B ratio. Differences in the ratio of

cy17:0/16:1 ω 7c were attributed to a positive effect of pH and a negative effect of soil moisture, while the ratio of cy19:0/18:1 ω 7c was best explained by an exponential decay function with pH. A small but significant (p-value = 0.001) improvement was observed when a positive effect of clay content was included in this model.

In the PCA, the first two axes explained 53 % of the variance in the correlation structure for the relative abundance of the individual fatty acids (**Figure 3**). PC axis 1, generally separated firmicutes, actinobacteria, and AMF biomarkers (left side of ordination) from SapF biomarkers and general bacteria biomarkers (right side of ordination). A linear regression model including negative effects of pH and silt content explained 86 % of the variance along this axis (**Table 3**). PC axis 2 generally separated firmicutes and general bacteria biomarkers (top of ordination) from gram⁻ and fungi biomarkers (bottom of ordination). Fifty-seven percent of the variance along PC axis 2 could be explained by a linear negative effect of the mean organic horizon depth, and linear positive effects of soil moisture and sand content. However, regression modeling also revealed that a non-linear, unimodal model with pH and a secondary positive effect of soil moisture (as Θ_v) could also be used to explain 73 % of the variance along this axis.

3.2 Microbial Communities across a Landscape Context Gradient

3.2.1 Plot-Level Analysis

First, we wanted to establish differences in microbial measures across our three landscape context - soil horizon subgroups (i.e., F-O, F-M, and M-M), in order to differentiate between soil horizon and landscape context effects. A number of significant differences emerged in these two-way ANOVAs for both edaphic properties (**Table 4**) and microbial measures (**Figure 4**). Edaphic properties with soil horizon effects included: SOM content, Θ_v , soil extractable N-NH₄⁺ concentrations, and Litter_{TC}. Horizon effects were also found for many

microbial abundances measures, including: gram⁻ bacteria, firmicutes, actinobacteria, and SapF, as well as the F/B ratio and the cy17:0/16:1 ω 7c ratio (**Figures 4.a-d, 4.f, and 4.h**). All of these edaphic properties and microbial measures were higher in F-O plots compared to M-M and F-M plots, except for Θ_v and the cy17:0/16:1 ω 7c ratio, which were higher in F-M and M-M plots compared to F-O plots. A landscape context effect was found for Θ_g , soil extractable N-NO₂⁻+NO₃⁻ concentrations, soil texture properties and Litter_{Biomass}; Θ_g , sand content, and Litter_{Biomass}, were higher in F-O and F-M plots compared to M-M plots, and soil extractable N-NO₂⁻+NO₃⁻ concentrations, and silt and clay content were higher in M-M plots compared to F-O and F-M plots. Both horizon effects and landscape context effects were found for pH, where the highest values were found in M-M plots, and the lowest values were found in F-O plots. Similar effects were found for AMF biomarker abundance and the cy19:0/18:1 ω 7c ratio, but in variable patterns; AMF biomarker abundance was highest in F-O plots and lowest in F-M plots (**Figure 4.e**), while the cy19:0/18:1 ω 7c ratio was highest in F-O plots and lowest in M-M plots (**Figure 4.g**).

Significant effects were found for microbial composition analyses. F-O and F-M plots were separated from M-M plots along PC axis 1, while F-O plots were separated from F-M plots along PC axis 2 (**Figure 3**). We also found effects when evaluating the relative abundance of major biomarker groups (**Figure 5**). We found horizon effects for the relative abundance of firmicutes and actinobacteria; the relative abundance of both these gram positive (gram⁺) bacteria groups were higher in F-M and M-M plots than in F-O plots. A landscape context effect was found for the AMF biomarker, where M-M plots had higher relative abundances than F-M and F-O plots. Both horizon effects and landscape context effects were found for gram⁻ bacteria and SapF biomarker groups, but in different patterns. The relative abundance of gram⁻ bacteria

was highest in F-M plots, and lowest in F-O plots, while the relative abundance of SapF was highest in F-O plots and lowest M-M plots.

3.2.2 Site-Level Analysis

Second, we used microbial measurements from PLFA profiling sites and modeled microbial measurements from the regional edaphic dataset sites to look for relationships between landscape metrics and microbial measures. Using these predicted microbial measures explicitly describes these distal controls as they are mediated through the proximal edaphic conditions described in each model. We first evaluated the regional edaphic dataset sites as a reflection of the entire Ridge and Valley physiographic province. The collective percent area of LULC categories across the regional edaphic dataset sites was generally reflective of the percent area of LULC categories across the entire extent of the Ridge and Valley physiographic province, and the extent of the physiographic province within Pennsylvania; the Ridge and Valley region is dominated by forest cover (i.e., ecoregion ~ 56 % (Friesen and Stier, 2005), Pennsylvania ~ 59 %), followed by agricultural land uses (i.e., ecoregion ~ 56 % (Friesen and Stier, 2005), Pennsylvania ~ 29 %), and only has small percentages of urban and commercial lands. The collective percent area of forest and agricultural LULC categories across the regional edaphic dataset sites were 65 % and 21 %, respectively.

The LDI values spanned the range of possible values, with an index of 1.00 equating to sites in 100 % natural landscapes, and an index of 8.54 equating to sites in ~100 % commercial lands. However, 92 % of headwater riparian wetlands within the regional edaphic dataset had values between 1.00 and 5.00, as reflected by the low levels of urban and commercial LULC within the region. Elevation ranged from 136 and 640 m for headwater riparian wetlands in the

regional edaphic dataset, constituting the lower end of the elevation gradient reported (i.e., 153 to 1,311 m) for the Ridge and Valley physiographic province (Friesen and Stier, 2005).

Edaphic conditions of the PLFA profiling sites were well within the conditions of the regional edaphic dataset sites. Clay content, pH, and soil moisture (as Θ_v) were well represented by the range of plot-level samples collected for PLFA profiles in comparison to the regional edaphic dataset (**Figure 6**). PLFA profile plots did not reflect the full extent of soil conditions found for SOM content, sand content, and silt content in the regional edaphic dataset. However, only 7 of 399 (1.8 %) samples and 37 of the 399 (9.2 %) samples taken across the 87 regional edaphic sites had higher or lower SOM contents (respectively), compared to the PLFA profiling sites. Only 3 of 87 (3.4 %) samples across 24 sites had higher sand contents, and only 9 of 87 (10.3 %) samples across 24 sites had lower silt contents. It should be noted that 51 % of the samples in the regional edaphic dataset that were outside the bounds of edaphic conditions found in the PLFA profiling plots came from headwater riparian wetlands with ≥ 90 % forest cover in their landscape assessment areas.

We then looked for relationships between microbial measures and landscape metrics using simple Mantel's tests or partial Mantel's tests when spatial autocorrelation was present for a variable in the analysis (**Table 5**). Among site similarity in PLFA abundance measures (i.e., individual biomarkers, total PLFA abundance, and community abundances of biomarkers), were not significantly related to among site similarity in percent area LULC metrics, the landscape percent area composition metric, or the LDI metric. Among site similarity in PLFA abundance measures were significantly related to among site similarity in wetland elevation. A plot of the raw data between total PLFA abundance and elevation, suggests a significant positive association (e.g., Total PLFA abundance in: **Figure 7.a**, $n = 31$, Adj. $R^2 = 0.33$, $p\text{-value} < 0.001$).

Elevation most directly explained the high among-site variability in total PLFA abundance measures (**Figure 7.b**) for headwater riparian wetlands in highly forested landscapes (i.e., percent forest area > 90 %), which span much of the wetland elevation gradient; headwater riparian wetlands in mixed landscapes were confined to lower elevations and as such, elevation and landscape composition were cofounded.

Although there were no significant associations for community biomarker relative abundances, associations were evident for other microbial composition measures. For example, among site similarity in the cy19:0/18:1 ω 7c ratios and PC axis 1 values were significantly associated with among site similarity in percent area of row crops, pasture and grasslands, and forests, as well as the landscape percent area composition metric and the LDI metric. Plots of the raw data for these microbial composition measures show significant negative associations with the LDI metric (**Figure 8.a**, $n = 26$, Adj. $R^2 = 0.58$, $p\text{-value} < 0.001$, and **8.c**, $n = 26$, Adj. $R^2 = 0.62$, $p\text{-value} < 0.001$), percent area of row crops, and pastures and grasslands (**Appendix A. Supplementary Data: Figure S2**), and significant positive associations with percent area forest (**Figure 8.b**, $n = 26$, Adj. $R^2 = 0.70$, $p\text{-value} < 0.001$, and **8.d**, $n = 26$, Adj. $R^2 = 0.68$, $p\text{-value} < 0.001$).

Among site similarity in the cy17:0/16:1 ω 7c ratio was also associated with among site similarity in percent area of pasture and grasslands, percent area of forest, the LDI index, and wetland elevation. However, plots of the raw data showed relationships opposite that of the cy19:0/18:1 ω 7c ratio; the cy17:0/16:1 ω 7c ratios had positive relationships with the LDI index (**Figure 8.e**, $n = 20$, Adj. $R^2 = 0.35$, $p\text{-value} < 0.001$) and percent area pasture and grasslands (**Appendix A. Supplementary Data: Figure S2**), and a negative relationship with percent area forest (**Figure 8.f**, $n = 20$, Adj. $R^2 = 0.49$, $p\text{-value} < 0.001$) and elevation (**Appendix A.**

Supplementary Data: Figure S2). Among site similarity in cy17:0/16:1 ω 7c ratio was also associated with among site similarity in wetland elevation (**Appendix A. Supplementary Data: Figure S2).**

Soil moisture data wasn't unavailable for most of the regional edaphic dataset sites; as such, we used soil pH, alone to model PC axis 2 ($n = 42$, $R^2 = 0.64$, Eq: $PCA_{axis2} = 41.9 \cdot pH - 6.6 \cdot pH^2 + 3.4e^{-01} \cdot pH^3 - 83.7$). PC axis 2 relationships were much weaker than for the cy19:0/18:1 ω 7c ratio and PC axis 1 values. An ordination plot of the PC axes, including percent area forest cover denoted by the size of points, suggested that landscape context separation holds across the regional edaphic dataset primarily along PC axis 1 (**Appendix A. Supplementary Data: Figure S3).**

4. Discussion

4.1 Microbial Habitat Models

SOM content had the strongest association with all PLFA abundance measures and with F/B ratios. These results were expected, as microbes are not only a small labile component of this pool, but SOM acts as a dominant carbon and energy source for microbes. This finding is consistent with other correlative and causal studies relating soil carbon to PLFA microbial biomass across an array of ecosystems (Allison et al., 2007; Anderson and Domsch, 1989, 1985; Anderson and Joergensen, 1997; Ushio et al., 2008; Wardle, 1992; Yao et al., 2000). Additionally, correlations between microbial biomass carbon and both SOM content and soil carbon have been found in wetland soils across anthropogenic disturbance gradients (Dinesh et al., 2004; Rokosch et al., 2009). The PLFA microbial abundances of organic horizon samples were higher than levels reported elsewhere for floodplain soils (D'Angelo et al., 2005; Langer

and Rinklebe, 2009; Rinklebe and Langer, 2010). However, these studies focused on mineral soils (i.e., A horizon) and microbial abundance estimates in our study were comparable to soils of similar SOC content (Bååth and Anderson, 2003). PLFA microbial abundance estimates were also lower or equivalent to estimates in forest humus layers (Leckie et al., 2004).

Although relatively small in comparison to SOM content effects, soil texture significantly improved the total PLFA abundance model and bacterial abundance models, with positive or negative relationships with clay and sand content, respectively. Positive relationships have been reported between clay content and microbial biomass (Gregorich et al., 1991; Merckx et al., 1985; Van Veen et al., 1985). This relationship has predominantly been attributed to the ability of clay particles to attach organic materials, buffer changes in pH, and protect microbial cell walls (Jiang-shan et al., 2005). Other studies have shown direct relationships between pH and PLFA abundance (Anderson and Joergensen, 1997; Bååth and Anderson, 2003; Högberg et al., 2007). However, these studies had much narrower ranges of soil organic carbon (SOC) (e.g., 3.5 to 8.9 % from Bååth and Anderson, 2003; 2.6 to 11.1 % from Anderson and Joergensen, 1997) than estimates of SOC from our PLFA profile sites (i.e., 2.3 to 32.0 % using Ranney, 1969 equation: $\text{SOC \%} = (\text{SOM \%} - 0.35) / 1.80$ for surface soils of Pennsylvania, or 2.6 to 33.6 % using David, 1988 equation: $\text{SOC \%} = (\text{SOM \%} / 1.72)$ for forest soils). In addition, soil texture cannot be disentangled from soil pH, which was significantly correlated with clay ($df = 40$, $r = 0.67$, $p\text{-value} < 0.001$) and sand content ($df = 40$, $r = -0.56$, $p\text{-value} < 0.001$).

In this study pH was initially selected during stepwise regression as a predictor of F/B ratios, but was removed because it did not significantly improve the model fit once SOM and Litter_{TN} were added to the model. Fungi are less sensitive to changes in pH than bacteria and as such are favored in lower pH soils. However, current literature has no consensus on the

relationship between F/B ratios and soil pH. Some studies using selective inhibition techniques to estimate microbial biomass, have shown a decrease in the F/B ratio with increasing pH (Bååth and Anderson, 2003; Blagodatskaya and Anderson, 1998). Conversely, other studies using PLFA to estimate biomass have reported little change or a slight increase in the F/B ratio with increasing pH (Bååth and Anderson, 2003; Bååth et al., 1992; Frostegård et al., 1993). In our study the relationship between pH and the F/B ratio followed an exponential decay ($n=42$, Eq: $F/B \text{ ratio} = 0.32 + 7203.01 \cdot \text{Exp}[-2.93 \cdot \text{pH}]$, $R^2 = 0.64$), with a significant drop between pH levels of 3 and 4. Soil pH was also a predictor of AMF abundance, which has been found across a range of mineral and humus soils layers in terrestrial forested ecosystems (Bååth and Anderson, 2003; Bååth et al., 1995, 1992).

Soil pH could also be used to explain variability in the relative abundances of individual fatty acids, such as cy17:0, 16:1 ω 7c, and cy19:0 in ordination space, and the cy17:0/16:1 ω 7c and cy19:0/18:1 ω 7c ratios. An increase in cyclopropyl fatty acids has been related to various environmental stressors (Bååth and Anderson, 2003; Guckert et al., 1986; Knivett and Cullen, 1965; Law et al., 1963; Petersen and Klug, 1994; Pietikäinen et al., 2000; Ratledge and Wilkinson, 1988; Thomas and Batt, 1969). However, interpretations of ratio results should be taken with some level of caution. Cyclopropyl fatty acids (cy17:0 and cy19:0) are produced by transmethylation of cis-monounsaturated fatty acids as cells enter a stationary phase, where loss of lipids and changes in membrane fluidity can be minimized during stressful conditions (Guckert et al., 1986). However, changes in these ratios could also be due to shifts in species composition (Frostegård et al., 2010).

Carbon availability is one potential stressor of these gram⁻ bacteria. Higher rates of carbon addition to soils are expected to shift proportions from gram⁺ bacteria (i.e., actinomycetes

and firmicutes) to fungi and gram⁻ bacteria (Griffiths et al., 1999). As such, Fierer et al. (2003) has related lower carbon content with soil depth to a rise in gram⁺ bacteria and decline in gram⁻ bacteria. Soil pH, could in part, act as a stressor to gram⁻ bacteria by limiting the availability of carbon, as was found by Persson et al. (1989) when acidifying humus soil layers of spruce and pine forest stands. Knivett and Cullen (1965) found an increase in the percentages of cy17:0 and cy19:0 fatty acids in isolates of *Escherichia coli* with acidities less than 6.2 and 5.5, respectively. Similarly, Bååth and Anderson (2003) and Bååth et al. (1992) found significant negative relationships between soil pH and the relative abundance of cy19:0 in soils, but also found significant positive relationships between soil pH and 16:1 ω 7c (Bååth and Anderson, 2003) and cy17:0 (Bååth et al., 1995, 1992) fatty acids. Our study followed patterns found in the latter papers; the positive relationship found between pH and the cy17:0/16:1 ω 7c ratio could be explained by the degree of correlation between pH and the cy17:0 fatty acid vs. the degree of correlation found between pH and the 16:1 ω 7c fatty acid.

Differences in the cy17:0/16:1 ω 7c ratio could also be driven by stressors other than pH, as was suggested by the equally important relationship between this ratio and a decline in soil moisture. Studies have reported that reduced soil environments, associated with prolonged high soil moisture, are associated with lower estimates of PLFA biomass (Bossio and Scow, 1998; D'Angelo et al., 2005; Langer and Rinklebe, 2009; Unger et al., 2009) and shifts in microbial abundances from aerobically associated fungi (Drenovsky et al., 2004; Langer and Rinklebe, 2009; Mentzer et al., 2006) and gram⁻ bacteria to gram⁺ bacteria (Balasooriya et al., 2008). Gram⁺ bacteria are known to have a strong association with anaerobic conditions (Sundh et al., 1997) prevailing in wetlands and increasing with soil depth, while gram⁻ bacteria are known to associate with aerated conditions (Ponder Jr. and Tadros, 2002). However, with their more

complex cell walls and their high resource demands required for synthesizing osmotic protectants, gram⁻ bacteria are also poorly adapted to aerated soils in drought conditions (Schimel et al., 2007). Using a slightly modified stress index (i.e., bacterial stress index = $\text{cy17:0} + \text{cy19:0} / 16:1 \omega 7c + 18:1 \omega 7c$), Gray et al. (2011) also found significantly higher levels of cyclopropyl to cis fatty acids in dry treatments ($\Theta_v = 21.7\%$ in top 15 cm) compared to wet treatments ($\Theta_v = 26.2\%$ in top 15 cm) of floodplain alluvium soils (treatments were at ambient CO₂ levels).

4.2 Linking Microbial Measures to Landscape Metrics

Microbial abundance measures were linearly related to landscape position, as described by wetland elevation, which is highly related to SOM content. Soil texture, while included in the microbial abundance habitat models, played a much smaller role in microbial abundance predictions in the regional edaphic dataset sites. In fact, a larger effect of soil texture would have led to a ‘cancelling out’ of any relationship between microbial abundance and elevation since, across our headwater riparian wetland sites, clay content tends to decrease (i.e., linear regression model, $n = 18$, Adj. $R^2 = 0.32$, $p\text{-value} < 0.01$) with elevation. Our findings are counter to some wetland and riparian studies, which have found a decrease in SOM content with elevation (Stolt et al. 2001; Wei et al. 2008; Xiong et al. 2015). This negative relationship is commonly attributed to higher erosion in more steeply sloped areas (Xiong et al. 2015), with subsequent transport of sediment and carbon sources downslope (Wei et al. 2008), as well as to soil wetting in flatter, downslope locations (Stolt et al. 2001).

In contrast, positive trends between SOM pools and elevation have been reported in forested regions by Garten et al. (1999), in the southern Appalachian Mountains, U.S., and more recently by Griffith et al. (2009) in the Cascade Mountains, OR, U.S. In these studies SOM was

linked to lower temperatures (Garten et al. 1999; Griffith et al. 2009) and higher soil moisture (Griffith et al. 2009) at higher elevations, both of which can slow decomposition rates. However, in both cases elevations spanned a much larger range (i.e., 235 to 1670 m, and 500 to > 1500 m, respectively) than in our study (136 and 640 m). While soil moisture is a controlling factor across our elevation gradient, the limited elevation range of our sites (and thus limited temperature range) suggests that variability in soil moisture is, instead, attributable to regional-scale geomorphology and attendant hydrology and soil characteristics as one moves from upper elevation, ridge environments to lower elevation, valley floor environments. Lower elevation wetlands are more likely to have larger upslope watershed areas, and thus greater hydrologic inputs, than their higher elevation counterparts. Visual inspection of the headwater riparian wetlands used in this study supported this effect; higher elevation wetlands tended to be primarily groundwater fed, while lower elevation wetlands were situated in more active floodplains, with greater surface water inputs (personal communication, Susan Yetter, Riparia, PSU). Active floodplains generally exhibit larger hydrologic fluctuations compared to groundwater fed wetlands (Mitsch and Gosslink, 2000), which can increase scouring and sedimentation rates (Wardrop and Brooks, 1998). Higher sedimentation rates can lead to an increase in soil bulk density (Koning, 2004; Werner and Zedler, 2002), and subsequent reductions in soil water storage capacity (Boelter, 1964). Overall, the drier conditions in lower elevation sites could enhance oxidation of SOM, while the wetter, hydrologically stable conditions found at higher elevation groundwater-fed sites could slow decomposition, favoring the accrual of carbon sources. These elevation-related factors, and their influence on SOM, are particularly important in explaining the variability in PLFA abundance measures in the highly forested sites (**Figure 7.b**), which occurred across the elevation gradient (**Figure 7a**).

However, elevation is not an appropriate predictor across all sites because of conflation between landscape position and landscape composition in this study. While ‘natural’ (i.e., forested) landscape wetlands (i.e., forest cover > 90 %, LDI < 1.5) spanned much of the wetland elevation gradient, mixed landscape wetlands (i.e., forest cover < 86%, LDI > 1.5) existed only at lower elevations. This has been problematic for other regional-scale studies attempting to tease apart landscape position and landscape composition effects in riparian zones (e.g., Xiong et al. 2015), and stems from the nonrandom distribution of anthropogenic uses more common in flatter, lower elevation terrain. Lower elevation and gentler terrain metrics have previously been used to explain why areas were more likely to remain in non-forest LULC categories in the lower Appalachian region (Turner et al. 1996, Wear and Bolstad 1998) after extensive forest clearing at the turn of the 20th century (Williams 1989). Thus, for mixed landscape wetlands, factors related to elevation do not adequately predict SOM content, and consideration of other factors is necessary.

In fact, lower mean levels of SOM content in surface soils of ‘degraded’, restored and created wetlands compared to ‘reference standard’ sites have previously been reported for this region and elsewhere (Bishel-Machung et al., 1996; Bruland and Richardson, 2005; Bruland et al., 2003; Campbell et al., 2002; Moon and Wardrop, 2013), and have been postulated to reflect water table depth and hydrologic stability (which affects soil moisture and thus soil decomposition rate) that are affected by distal controls of surrounding landscape composition. A recent study (i.e., 2010 and 2013) that included headwater riparian wetlands in urban, agricultural, and forested landscapes in Pennsylvania (including many sites from this study) and Ohio, reported that the percent time that the water table remained within the top 30 cm of soil was higher for wetlands with lower LDI indices than those with higher LDI indices in a 200 m

radius landscape assessment area (Wardrop et al., In Press). Although Wardrop et al. (2016) did not control for wetland elevation in their assessment, other studies have shown similar patterns (e.g., Groffman et al., 2002). Further, our study supports these findings with higher soil moisture (as Θ_g) conditions found in PLFA profiling sites in forested landscapes compared to those in mixed landscapes.

In addition to hydrologic factors, sedimentation and nutrient inputs at the site-scale could explain the lower SOM content found in lower elevation mixed landscape sites. Mixed landscape sites are expected to receive elevated sediment deposits (Wardrop et al., In Press; Johnston et al. 1984; Hupp et al. 1993; Hupp and Bazemore 1993; Kieiss 1996, Wardrop and Brooks, 1998). We found lower elevation forested landscape sites to have significantly lower silt contents compared to their mixed landscape counterparts (i.e., Kruskal-Wallis Test, low elevation forested landscape sites = 11, low elevation mixed landscape sites = 7, $X^2 = 6.2$, p-value = 0.0128). These inputs should favor the influx of available nutrients (Hupp et al., 1993) and “hot moments” of high mineralization rates (Birch, 1958; Cui and Caldwell, 1997). This was supported by the higher extractable $N-NO_2^-+NO_3^-$ concentrations found in PLFA profiling sites in mixed landscapes compared to those found in forested landscapes.

Due to land-use legacies, on-site vegetation is also expected to vary between forested and mixed landscapes sites, irrespective of an elevation gradient. Much of the region was logged in the late 1800s, and forested landscape sites were left to re-establish after logging; we found no significant differences in the percent basal area of tree species across regional edaphic sites in forested landscapes as a function of elevation (i.e., $n = 17$, p-value = 0.46, Mantel's $r = 0.0036$). However, mixed landscape sites had a range of land-use legacies (e.g., agricultural practices, residential lawns, filled stream channels) that varied from site to site and in duration. While

there was an overall net loss in forested cover in the eastern U.S. between 1973 and 2000, including in the Ridge and Valley physiographic province (i.e., - 1.5 % area loss), there has also been a gain in forest cover due to the abandonment of agricultural lands during this period (Drummond and Loveland 2010). Using historical aerial photographs, the PLFA profiling sites in mixed landscapes have been documented as a part of this forest expansion (Moon and Wardrop 2013). This study revealed that headwater riparian wetlands in mixed landscapes have higher percentages of herbaceous cover compared to their forested landscape counterparts (Moon and Wardrop, 2013).

Thus, mixed landscape sites lag behind in their vegetative successional development, and these vegetative differences can explain differences in decomposition rates through variation in substrate sources and microclimate (e.g., temperature, moisture). The higher Litter_{Biomass} and Θ_g found in plots of forested vs. mixed landscape sites, are also suggestive of such effects. The effects of microclimate on the control of humus depth have been established in tree gaps of forested systems (Podrázský and Remeš, 2006; Waez-Mousavi et al., 2012). D'Angelo et al. (2005) calculated that it could take up to 130 years for early successional wetlands to reach levels of SOC found in their late successional counterparts, although they warn that this might be an overestimate for wetlands, given the rates used in this calculation were developed in well-drained forest soils.

There were also significant associations between microbial composition measures and landscape composition metrics, although significant correlations among landscape metrics make it difficult to discern any possible cause and effect relationships. In this study, we were able to determine that more complex landscape metrics, such as the landscape percent area composition metric and the LDI index, did not outperform simpler percent area LULC category metrics. We

recognize that this may be due to low heterogeneity of LULC categories in this region (i.e., primarily, forest and agriculture). In other words, this result might be less applicable to regions where the landscape is more equally distributed across a larger range of LULC categories (e.g., higher percent area covers of commercial and urban lands), where metrics such as the LDI index are evenly distributed across their full potential range.

The strongest relationships between landscape metrics and composition measures were found for the first PC axis derived from individual biomarkers and the $cy19:0/18:1\omega7c$ ratio. Both of these microbial measures were most strongly associated with percent area of agricultural LULC categories (i.e., row crops, pasture and grassland) and percent area of forest. Soil pH and soil texture mediated these distal controls. We found that sand content was higher in forested landscape sites compared mixed landscape sites, regardless of elevation. Drohan and Brooks (2013) have suggested that lower sand content in ‘disturbed’ wetlands compared to ‘reference standard’ wetlands, is likely due to erosional inputs of silt and clay from mixed landscapes.

Further, higher pH levels in soil and water have previously been associated with legacy effects and surrounding land-uses in a number of wetland studies (Bruland and Richardson, 2005; Cleveland et al., 2011; Cohen et al., 2008; Reiss, 2006). The sandy mineral soils, common in forested landscape sites (regardless, of elevation) sit in close proximity to organic horizon soil patches with high SOM content. This SOM contains a number of acid functional groups and tends to acidify soils by forming soluble complexes with base cations that are then leached (Brady and Weil, 2002). These mineral soils also have a lower pH buffering capacity compared to clay and silt soils, and as such might be affected by acidic rain, common to the region (Likens, 2010).

One of the strongest separations in biomarker groups along PC axis 1 were the relative abundances of fungal communities. Higher relative abundances of saprophytic fungi were found in natural landscape sites, while higher relative abundance of AMF were found in landscapes with a predominance of agricultural land-uses. Although there wasn't a clear distinction in Litter_{TC} between forested and mixed landscape sites, these differences could be attributed to the types of carbon sources most commonly available across sites. Forested landscape headwater riparian wetlands have been reported to have higher abundance of coarse woody debris (Wardrop et al., 2007a; Brooks and Gebo, 2013) commonly used by saprophytic fungi (De Deyn et al., 2008)), while the mixed landscape wetlands in this study have been associated with higher percentages of herbaceous cover (Moon and Wardrop, 2013), commonly used by AMF (De Deyn et al., 2008).

In terrestrial systems, AMF are associated with improving phosphorous acquisition for their plant hosts by extending their hyphae and capturing phosphorus in zones inaccessible to the plant (Smith and Read, 1997). A meta-analysis of agricultural and forested ecosystems by Treseder (2004) showed a 15 and 32 % decline in mycorrhizal fungi abundance with nitrogen and phosphorus fertilization. Wetland studies have further suggested that the rate of AMF colonization might be lower in high fertility soils (Wetzel and van der Valk, 1996) or in, inundated sites with high phosphorus availability (Stevens et al., 2002) or higher water table depth (Miller and Bever, 1999). In our study the opposite effect was found; AMF abundance was positively correlated with soil phosphorus ($n = 41$, $R^2 = 0.58$, $p\text{-value} < 0.001$), soil extractable $N\text{-NH}_4^+$ ($n = 37$, $R^2 = 0.50$, $p\text{-value} < 0.001$), and Θ_g ($n = 42$, $R^2 = 0.07$, $p\text{-value} = 0.04$). However, this could be due to the confounding effect of SOM content, which was correlated with both AMF abundance and these soil properties (results not shown). In addition

AMF relative abundance was not correlated with soil phosphorus or extractable N-NH₄⁺, but was negatively correlated with Θ_g ($n = 42$, $R^2 = 0.28$, $p\text{-value} < 0.05$).

4.3 Conclusion

In this headwater riparian wetland study, we have shown strong links between microbial biomass and composition measures and combinations of edaphic properties, primarily including SOM content, soil pH, soil moisture and Litter_{TN}. These strong relationships, along with high within-site variability at the PLFA profiling sites, allowed us to extrapolate microbial measures to a regional scale. Doing so allowed us to highlight the utility of landscape metrics as indices of edaphic conditions and associated microbial abundance and composition in headwater riparian wetlands. These landscape metrics could be useful in predicting changes in microbial community composition of headwater riparian wetlands with future LULC changes, and thus provide an indication of the level of biogeochemical-related ecosystem services that could be expected. Paired PLFA and stable isotope analyses are expected to help tease out the contribution of microbes to differences in ecosystem functioning (Watzinger, 2015), and provides a next step for this research.

The landscape assessment area used in this study (200-m radius circle) is much smaller than has been previously used for wetland condition assessment protocols in Pennsylvania (1-km radius buffer, Wardrop et al., 2007b). However, Mack (2006) suggested that the use of areas < 1 km could improve linearity between landscape metrics (e.g., LDI index) and measures of ecological conditions in wetlands, as smaller buffers are expected to reflect distal controls more directly. Our linear relationships between microbial composition measures and landscape composition metrics support this claim. In a comprehensive literature review by Castelle et al.

(1994), the 200-m radius assessment area was established as the upper limit of the effective buffer range for wetlands and streams to ameliorate effects arising from a range of stressors (e.g., sediment and nutrient removal, erosion and storm water runoff, water temperature control, habitat and species distribution and diversity). As we focused our attention on a fixed landscape assessment scale, further work will be needed to characterize the effective buffer range for identifying changes in microbial composition measures, most specifically those that affect organic horizon formation, which in this study is the primary driver of microbial abundance measures.

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PLFA Sites

Regional Sites

Chesapeake Watershed

Deciduous Forest

Evergreen Forest

Mixed Forest

NWI Forested Wetland*

NWI Emergent Wetland*

Pasture / Grassland

Row Crop

Water

Bare

Residential

Commercial / Industrial

0 20 40 80 120 160 Kilometers

Wetland Assessment Area (2,025 m²)

Landscape Assessment Area (125,664 m²)

Sampling Plots (1 m²)

□ 1 m² Plots from Moon (2012)

■ 1 m² Plots selected for PLFA profiling and edaphic characterization (n = 2 - 10 per site)

● 1 cm² Soil Sampling Cores (n = 16 mineral and organic, when present, pooled)

○ 81 cm² Litter Sampling

Landscape Context - Soil Horizon Subgroups

- PLFA Dataset ● Regional Dataset – Mixed Landscape Sites
● Regional Dataset – Forested Landscape Sites

● PLFA Dataset ● Regional Dataset

Figure Captions

Figure 1. A map showing locations of headwater riparian wetlands used for phospholipid fatty acid (PLFA) profiling (filled circles, $n = 8$), and of headwater riparian wetlands where edaphic property information was available for predicting microbial measures across landscape metrics (open circles, $n = 79$). All headwater riparian wetlands were within the Ridge and Valley Region, denoted by land-use and land-cover categories from a 2005 PAMAP Digital Based Map (The Pennsylvania State University, 2007), of central Pennsylvania's Chesapeake Bay Watershed. *The National Wetland Inventory (NWI) forested and emergent wetland categories are present within the Ridge and Valley region. However, their extents are too small to be visible at the map resolution. At some wetlands these categories were found within the landscape assessment area ($2,025 \text{ m}^2$) used to calculate landscape metrics.

Figure 2. Sampling design used at headwater riparian wetlands ($n = 8$, each $2,025 \text{ m}^2$) where microbial habitat models were developed (i.e., phospholipid fatty acid (PLFA) profiling sites). At each site, 1-m^2 plots were selected from Moon (2012) to represent the range of edaphic conditions present in the wetland assessment area ($2,025 \text{ m}^2$). Within each of these plots edaphic property information and soil PLFA profiles were collected by pooling litter ($n = 4$), and soil cores ($n = 16$). Five cm of mineral soil were collected from each sampling point, and pooled per plot. When present, the complete organic horizon was collected from each sampling point and pooled per plot. At all PLFA profiling plots, and at all additional headwater riparian wetlands where edaphic property information was available (i.e., regional edaphic dataset sites), landscape metrics were calculated in a landscape assessment area ($125,664 \text{ m}^2$).

Figure 3. Principal components analysis (PCA) ordination of microbial community composition (mole %) using 23 individual phospholipid fatty acid (PLFA) biomarkers. Organic and mineral horizon plot samples (n = 42) are represented by dark brown fill and light brown fill, respectively. Forested landscape headwater riparian wetland plots (i.e., 90 – 100 % surrounding forest cover), and mixed land-use and land-cover landscape headwater wetland plots (i.e., 24 – 54 % surrounding forest cover) are represented by circles and triangles, respectively.

Figure 4. Boxplots for the phospholipid fatty acid biomarker abundances (nmol · g⁻¹ soil), including: **a.** gram negative bacteria (Gram⁻), **b.** firmicutes, **c.** actinobacteria (Actino), **d.** saprophytic fungi (SapF), and **e.** arbuscular mycorrhizal fungi (AMF), as well as the ratios of: **f.** fungi / bacteria (F/B), **g.** cy19:0a / 18:1ω7c, and **h.** cy17:0 / 16:1ω7c. Plots were grouped into forested landscapes with organic horizon samples (F-O: 90 – 100 % surrounding forest, organic surface horizon, n = 11), forested landscapes with mineral horizon samples (F-M: 90 – 100 % surrounding forest cover, mineral surface horizon, n = 17), and mixed land-use and land-cover landscapes with mineral horizon samples (M-M: 24 and 54%, surrounding forest cover, mineral surface horizon, n = 14). Letters denote significant differences (p-value < 0.05) between or among groups using two-way analysis of variance tests. Boxplot horizontal lines represent means, diamonds represent medians, and black dots represents outliers. Organic and mineral horizon soil samples are further represented by dark brown fill and light brown fill, respectively.

Figure 5. Relative abundance (mole %) of phospholipid fatty acid (PLFA) biomarkers for gram negative bacteria (Gram⁻), firmicutes, actinobacteria, saprophytic fungi, and arbuscular mycorrhizal fungi (AMF). Plots were grouped into forested landscapes with organic horizon

samples (F-O: 90 – 100 % surrounding forest cover, organic surface horizon, n = 11), forested landscapes with mineral horizon samples (F-M: 90 – 100 % surrounding forest cover, mineral surface horizon, n = 17), and mixed land-use and land-cover landscapes with mineral horizon samples (M-M: 24 and 54 % surrounding forest cover mineral surface horizon, n = 14). Letters across each microbial group denote significant differences (p-value < 0.05) between or among groups using two-way analysis of variance tests.

Figure 6. Radial plot comparing ranges of soil properties from plots at phospholipid fatty acid (PLFA) profile sampling sites (n = 42) to plots at the regional edaphic dataset sites (soil organic matter content, SOM: n = 399, volumetric water content, Θ_v : n = 192, pH: n = 370, sand, silt, and clay content: n = 87). Axes range from 0 to 100 % for SOM, Θ_v , and soil texture properties. Axes range from 0 to 10 for pH. Regional edaphic sites data were gathered from published and unpublished sources (Bishel, 1994; Bishel-Machung et al., 1996; Auman, 2004; Williams-Tober, M.R. 2009; Cleveland et al., 2011; Drohan and Brooks, 2013; and Wardrop, unpublished data).

Figure 7. Plots of average site-level total phospholipid fatty acid (PLFA) abundances ($\text{nmol} \cdot \text{g}^{-1}$ soil) (n = 31) as a function of **a.** wetland elevation and **b.** percent area forest in the landscape assessment area (125,664 m^2). Standard error bars represent variability among plots within each site. Dark gray circles denote observed microbial measures from PLFA profiling sites, and green circles denote predicted microbial measures from regional edaphic dataset sites. Dark green circles denote sites in forested landscapes (i.e., 90 – 100 % surrounding forest cover) and light green circles denote sites in mixed landscapes (i.e., 24 – 54 % surrounding forest cover). Linear regression lines indicate a significant (p-value < 0.05) relationship. Light gray portions of the

regression lines indicate model extrapolation using the predicted microbial measures from regional edaphic dataset sites alone.

Figure 8. Plots of average site-level **a-b.** PC axis 1 values ($n = 26$), **c-d.** $\text{cy19:0a} / \text{18:1}\omega\text{7c}$ ratios ($n = 26$), and **e-f.** $\text{cy17:0} / \text{16:1}\omega\text{7c}$ ratios ($n = 20$) as a function of the Landscape Development Intensity (LDI) index, and percent area forest cover within the landscape assessment area ($125,664 \text{ m}^2$). Standard error bars represent variability among plots within each site. Dark gray circles denote observed microbial measures from PLFA profiling sites, and light gray circles denote predicted microbial measures from regional edaphic dataset sites. Linear regression lines indicate a significant ($p\text{-value} < 0.05$) relationship. Light gray portions of the regression lines indicate model extrapolation using the predicted microbial measures from regional edaphic dataset sites alone.