

Leaf-litter microbial communities in boreal streams linked to forest and wetland sources of dissolved organic carbon

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Abstract. Leaf-litter microbial activity is influenced by several stream characteristics that may be affected by alterations in watershed condition. However, there have been few studies and little direct evidence that leaf-litter microbial communities are affected by disturbance-induced watershed condition, particularly in boreal streams. To test this linkage, we compare the associations of stream physical–chemical characteristics with landscape features (e.g., percent wetlands, roads, riparian woody stem diversity), and leaf-litter microbial activity and structure in streams across varying disturbance-induced watershed conditions. Our findings suggest that the increased stream water conductivity associated with roads can have a negative impact on leaf-litter microbial extracellular enzyme activity associated with a decrease in the abundance of Betaproteobacteria. Wetlands and forests in contrast are important providers of dissolved organic carbon that stimulates the microbial, and in particular fungal, cycling of energy and nutrients. We present a novel and in-depth perspective of leaf-litter microbial communities as a critical link to our understanding and management of the influences of watershed condition on aquatic ecosystems.

Key words: allochthony; bacteria; decomposition; dissolved organic carbon; enzyme activity; hyphomycetes; leaf-litter; stream; watershed disturbance.

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INTRODUCTION

Leaf-litter microbial activity is influenced by several stream characteristics that may be affected by alterations in watershed condition. For example, previous studies have shown that microbial leaf-litter activity is stimulated by increased stream water temperature (Martínez et al. 2014), optimal pH (Clivot et al. 2013), moderately increased nutrient inputs (Woodward et al. 2012), algal byproducts (Kuehn et al. 2014), and dissolved organic matter that can be assimilated to supplement leaf-litter decomposition and satisfy stoichiometric requirements (Pastor et al. 2014). Microbial leaf-litter activity can also be negatively affected by metals and other contaminants (Ferreira et al.

2016), which may be exported by watershed runoff. It has been demonstrated that impervious surface cover in urban watersheds can disrupt denitrifying bacterial communities in stream sediments (Wang et al. 2011) and that forest cover can affect biofilm and sediment extracellular enzyme activity across streams in the United States (Hill et al. 2012); however, studies linking microbial activity to disturbance-induced watershed condition are limited.

Allochthonous dissolved organic carbon (DOC) is an important terrestrial–aquatic resource that supports aquatic food webs (Pace et al. 2004, Tanentzap et al. 2014). The quantity and quality of allochthonous DOC can be altered by climate change (Zhang et al. 2010) and natural and

anthropogenic watershed disturbance (Yamashita et al. 2011) that alter hydrology, watershed vegetation, and soil conditions. Aquatic microbial communities on leaf litter are central to understanding how changes in allochthonous DOC are linked to the aquatic food web because of their role in the incorporation of dissolved organic matter into the aquatic food web, and decomposition and conditioning of particulate organic matter (Bärlocher 2005, Findlay 2010).

We are not aware of any studies that link leaf-litter microbial activity coupled with fungal and bacterial community structure to disturbance-induced watershed condition, despite the obvious importance of these microbial communities to terrestrial–aquatic linkages.

There is a need to better understand how leaf-litter microbial activity and community structure are affected by watershed condition if we are to fully understand how food webs in aquatic ecosystems are linked to disturbance and recovery in terrestrial ecosystems. Here, we test how stream leaf-litter microbial activity is linked to watershed condition across varying degrees of disturbance from mining/urban industrial activity, wildfire, and forest harvesting, to relatively undisturbed watersheds. To test this, we compare the associations of key stream physical–chemical characteristics including DOC, nutrients (TP, TN concentration), ion concentrations (conductivity), temperature, pH, sedimentation, large woody debris (LWD), and metal contaminants (Ni) with measures of microbial activity and link these to watershed characteristics. We also explore associations between microbial activity and community structure using biomass estimates and DNA meta-barcoding of fungal and bacterial communities to help further understand the differences in microbial activity associated with land–water linkages. This expands on our previous work that has identified differences in leaf-litter microbial communities between streams with different disturbance histories (Emilson et al. 2016), now demonstrating the degree to which those differences are associated with specific watershed and stream characteristics. The identification of these key associations with watershed, stream, and microbial characteristics furthers our understanding of connections between terrestrial and aquatic ecosystems with potential to aid in restoration and management strategies.

METHODS

Study design

To test how microbial activity is linked to watershed condition, we collected leaf-litter microbial communities and measured stream and landscape features for 24 low-order boreal streams across differing watershed conditions. Differing watershed conditions were the result of either low to moderate disturbance from fire ($n = 7$) or logging activity ($n = 5$), high disturbance from industrial activity ($n = 3$) or industrial activity confounded by urban commercial and residential development ($n = 3$), or by no disturbance in at least the last 50 years ($n = 6$). Our interest was not in a comparison among disturbances but in using the different disturbance types to maximize differential boreal forest watershed conditions. All streams are located on the Boreal Shield of northern Ontario, Canada, surrounded by varying proportions of balsam fir (*Abies balsamea*), white and black spruce (*Picea glauca*, *Picea mariana*), white, red, and jack pine (*Pinus* spp.), white birch (*Betula papyrifera*), and trembling aspen (*Populus tremuloides*). Industrial and industrial–urban streams are located in the Greater Sudbury Area, and undisturbed, logged, and fire streams near White River (Fig. 1). Streams had comparable average depths of ≤ 0.3 m, and average velocities of ≤ 0.3 m/s, and varied in stream bottom composition (see Appendix S1: Tables S1 and S2 for ranges in stream and landscape characteristics).

Microbial activity and community structure

Six replicate fine mesh (0.5-mm) leaf packs containing senescent speckled alder leaves (*Alnus incana* ssp. *rugosa*) were incubated in each stream for 6 weeks during the summer of 2012. Leaf packs were incubated in the summer to increase microbial colonization potential by ensuring base flow conditions and slightly warmer water temperatures than in the fall (Maloney and Lamberti 1995, Villanueva et al. 2011). Standardized substrate was provided to remove the influence of variable leaf-litter composition and allow for the assessment of environmental influences on leaf-litter communities. Fine mesh was used to exclude macroinvertebrates and allow for estimation of microbial decomposition.

In each replicate leaf pack, a single 50 mm diameter leaf disk was included separately for the

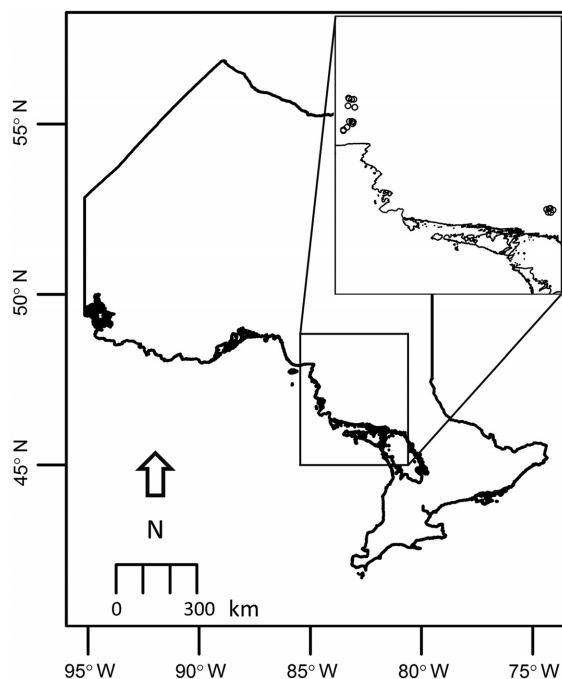


Fig. 1. Map of Ontario, Canada, showing location of 24 study streams.

estimation of microbial decomposition. Each replicate leaf pack also included approximately eight leaves for extracellular enzyme activity assays and 10 leaves for DNA extractions and fungal and bacterial biomass estimates. Leaf-litter decomposition was measured as the percent mass loss of the six replicate leaf disks over the six-week incubation period by measuring the dry weight of each leaf disk (dried at 30°C for 48 h) before and after incubation in the stream. Prior to incubation, the leaf material was pre-leached under running water before being cut into 50-mm disks, and after incubation, the leaf disks were gently washed prior to drying and weighing to remove any non-leaf-litter material (Bärlocher 2005). Potential extracellular enzyme activities were measured as outlined in Emilson et al. (2016) on a BioTek Synergy H1 Hybrid reader (BioTek, Winooski, Vermont, USA). The extracellular enzyme activities measured included α -glucosidase (α -G)—which breaks down simple starches— β -xylosidase (β -X)—which breaks down hemicellulose—leucine aminopeptidase (LAP)—a N-acquiring enzyme—phosphatase (PHOS)—a phosphorus-acquiring enzyme—and phenol oxidase (POX) and peroxidase (PER)—which break down more complex organic compounds such as

lignin. These enzymes were chosen based on their role in the breakdown of particulate and dissolved organic matter and subsequent cycling of C, N, and P (Sinsabaugh et al. 1994).

Fungal biomass estimates were based on solid-phase extractions and subsequent measurement of ergosterol concentrations on a HP1100 liquid chromatograph (Agilent, Hewlett Packard Canada Ltd., Mississauga, Ontario, Canada), and bacterial biomass estimates were done using ultrasonic probe sonication followed by flow cytometry cell counts on a BD FACSCanto II flow cytometer (Invitrogen, Carlsbad, California, USA). Detailed protocol for fungal and bacterial biomass estimates is outlined in Emilson et al. (2016). Additionally, DNA was extracted using a MO BIO PowerSoil DNA Isolation kit and protocol (MoBio, Carlsbad, California, USA) for each stream from a homogenized sample of the six replicates and sequenced using 454 pyrosequencing technology for fungal 18S (panfungal primer funSSUF 5'-TGGAGGGCAAGTCTGGTG-3') and bacterial 16S (universal bacterial 27F primer 5'-AGAGTTT-GATCMTGGCTCAG-3') communities at Mr.DNA's sequencing facilities in Shallowater, Texas. Raw sequence data have been deposited in the SRA under accession numbers SMAN03491913–SMAN 03491936 (BioProject PRJNA281310). After quality filtering and rarefaction (Emilson et al. 2016), the sequence data were filtered for the top three fungal and bacterial classes (see Appendix S1: Table S3 for ranges in all microbial community characteristics).

Stream physical–chemical characteristics

To associate the microbial communities with their stream environment, we measured select stream habitat features on a 50-m stretch of stream where the leaf packs were incubated. Physical stream habitat measurements were made in the summer of 2010 for undisturbed, fire and logged streams, and the summer of 2012 for industrial and industrial–urban streams. Remote study locations prevented re-assessment of all stream physical characteristics in 2012; however, all streams assessed in 2010 had a minimum of 5 years since disturbance, ensuring that all major disturbance-induced changes in stream physical condition would have already occurred during the first few years following disturbance. Measurements included stream depth, velocity,

streambed substrate composition (i.e., visual assessment of percent coarse cover including bedrock, boulder, rubble, cobble, and gravel; percent fine cover including sand and silt; and percent coarse organic matter cover), the abundance of large woody debris/m² (LWD), fine sediment deposition rates (g dry weight·m⁻²·d⁻¹), and percent organic matter in the sediment. Large woody debris pieces >10 cm in diameter and >1 m in length were counted. Fine sediment was collected in upright falcon tubes held in place by bricks, and then dried and weighed to calculate the amount of sediment (1.5-µm to 1-mm particles) deposited per unit area, and combusted at 500°C for 2 h to estimate the amount of organic material present.

Water chemistry analyses on mid-depth grab samples included DOC, dissolved inorganic carbon, nutrients (TP, TN), cations (Ca, Mg, Na, K), and metals and other anions (Ni, Cu, Fe, Mn, Cl). Either one or two grab samples were collected from the streams at base flow conditions during leaf pack incubation in the summer of 2012 and were analyzed in the Canadian Forest Service water chemistry laboratory following standard methods (Beall et al. 2001). Excitation–emission matrices were generated with an Agilent Cary Eclipse (Agilent Technologies, Santa Clara, California, USA), and the humification index (HIX) was calculated as a relative measure of the quality of dissolved organic matter (Zsolnay et al. 1999). Temperature and pH were measured in the field with a HI991003 portable pH meter (Hanna Instruments, Rhode Island, USA) and HI 1296 probe, and conductivity was measured in the field with a Primo 3 TDS Tester (Hanna Instruments).

Landscape features

We characterized landscape features at the watershed and riparian scale to determine the association with stream habitat and ultimately microbial activity and community structure. Watershed-level characteristics included percent productive forest, open wetland, open water, roughness (coefficient of variation in ground height across watershed), road density (road length m/watershed area m²), watershed area (m²), and drainage density (total stream length m/watershed area m²), and were calculated using ArcGIS software based on the White River Sustainable Forest management Plan 2008

(sfmm08), the Sudbury Forest Management Unit 2010 (889), and the Ontario Base Topographic Map. Watersheds were delineated using Digital Elevation Models and Enhanced Flow Accumulation Grids from Land Information Ontario.

Riparian shrub and juvenile tree surveys were conducted for each stream. Surrounding the 50-m transect where the stream habitat surveys were done, two 26-m transects parallel to the stream were marked 5 m from the stream edge on both sides for a total of four transects per stream. Along each transect, 50 cm diameter circular plots were examined every 2 m for a total of 14 circular plots per transect. The density (i.e., the number of stems originating from within each plot) for each species of woody stemmed vegetation was recorded for each plot and used to calculate the diversity of young growth comprising the understory (woody stems <10 cm). Young, understory growth, instead of larger, older growth, directly adjacent to the stream was used as a measure of the state of disturbance and recovery in the riparian zone.

Statistical analyses

Hierarchical partitioning complimented with redundancy analysis was used to explore associations between landscape features, the stream environment, and stream microbial communities across a variety of disturbance-induced watershed conditions. Our analytical pathway was to examine associations between microbial activity and stream physical–chemical characteristics, followed by associations between stream physical–chemical characteristics and surrounding landscape features, thereby linking microbial communities to their surrounding environment, and their stream environment to the surrounding landscape. The main response variable of interest in this study was microbial activity because of its importance and direct link to the cycling of energy and nutrients in the aquatic ecosystem. Measures of microbial community structure were also included in redundancy analysis to help better understand the possible mechanisms behind the observed associations between microbial activity and stream physical–chemical characteristics.

First, we used redundancy analysis to constrain measures of microbial activity and microbial community structure by stream physical–chemical

characteristics, and to constrain stream physical–chemical characteristics by landscape features. Redundancy analysis nicely compliments hierarchical partitioning by allowing for the exploration of associations between all response and explanatory variables simultaneously (Bertini et al. 2014). For each measure of microbial activity and associated stream physical–chemical characteristics, we then used hierarchical partitioning to calculate the individual influence of each explanatory variable. Hierarchical partitioning does this by calculating the individual and joint influence of each variable in all possible model combinations, and then taking the average (Chevan and Sutherland 1991, Mac Nally 2000). Hierarchical partitioning moves away from single model inferences and provides insight into the relative influence of each explanatory variable. Hierarchical partitioning and randomization testing using negative log-likelihood to calculate significance ($n = 1000$) were performed in the package *hier.part* (Walsh and Mac Nally 2013), and redundancy analysis in the package *vegan* (Oksanen et al. 2016) with permutation ANOVA to calculate the significance of each axis. Redundancy analysis (i.e., linear model) was chosen over correspondence analysis (i.e., unimodal model) based on initial detrended correspondence analysis that resulted in a beta diversity of <3 standard deviations (Van Wijngaarden et al. 1995).

All statistical analyses were run in R version 3.3.1 (R Core Team 2016). Prior to analyses, variables that were not normally distributed were transformed using logarithmic or square root transformations, or logit transformations for percentages. Explanatory variables were selected based on ecological theory and a variance inflation factor (VIF) cutoff of 5 to avoid problems of multicollinearity (O'Brien 2007). VIF was implemented in the *car* package for R (Fox and Weisberg 2011). In total, nine stream habitat variables with VIF values <5 were used and included pH, TP, TN, DOC concentration, Ni, conductivity, fine sediment deposition rates, LWD, and temperature. The landscape features used included Shannon diversity of riparian woody stem vegetation, percent productive forest cover, percent wetland cover, percent open water, the roughness coefficient, road density, and drainage density. These landscape features all had VIF values of <3.

RESULTS

Linking microbial communities to surrounding stream characteristics

The first two RDA axes of stream physical–chemical variables explained a total of 40.8% of the variation in microbial community activity and structure (Fig. 2). Based on a cutoff of >0.6, RDA1 was most strongly loaded by conductivity (biplot score 0.742) and sedimentation (biplot score 0.716), while PHOS (species score -0.745), β -X (species score -0.685), α -G (species score -0.659), and Betaproteobacteria (species score -0.649) were all negatively constrained by, and bacterial biomass (species score 0.678) positively constrained by, RDA1 across streams (Fig. 2). RDA2 was most strongly loaded by DOC (biplot score 0.639) and microbial decomposition (species score 0.787), while PER activity (species score 0.646), fungal biomass (species score 0.615), and POX activity (species score 0.604) were all positively constrained by RDA2 across streams (Fig. 2).

When looking at individual contributions of each stream physical–chemical characteristic on each measure of microbial activity based on hierarchical partitioning, DOC concentration on its own explained the most variation in microbial decomposition, and α -G, LAP, POX, and PER activities with independent contributions of 21.9%, 37.8%, 21.5%, 29.4%, and 28.4.0%, respectively, while conductivity on its own explained the most variation in β -X and PHOS activities with an independent contribution of 26.4% and 48.7%, respectively (Fig. 3). The above independent contributions of DOC and conductivity were all found to be statistically significant (negative log-likelihood randomization test $P < 0.05$) except for LAP activity, which had no statistically significant independent contributions from any of the stream physical–chemical characteristics (Fig. 3).

Linking stream characteristics to surrounding landscape features

Landscape features explained a total of 52.5% of the variation in stream physical–chemical characteristics across the streams in this study based on the first two axes of RDA (Fig. 4). On its own, RDA1 explained 38.1% of the variation and, based on a cutoff of >0.6, was most strongly

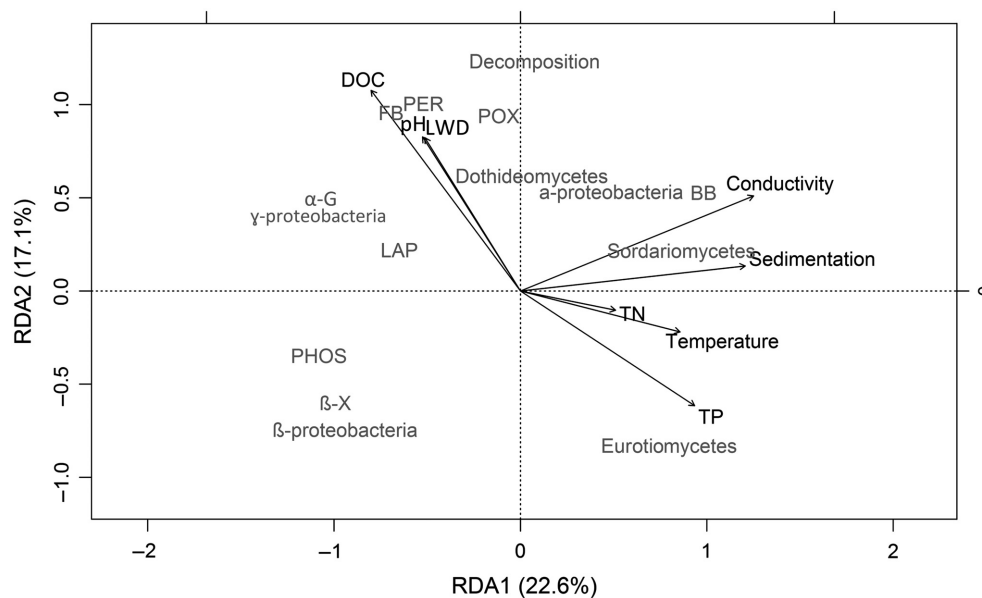


Fig. 2. Redundancy analysis showing associations between microbial community functional and structural characteristics (FB, fungal biomass; BB, bacterial biomass) and stream physical-chemical characteristics.

loaded with percent forest cover (biplot score 0.905) and road density (biplot score -0.679). Along RDA1, Ni (species score -1.09), TP (species score -0.913), temperature (species score -0.859), sedimentation (species score -0.813), conductivity (species score -0.760), and TN (species score -0.703) were all negatively constrained (Fig. 4).

Based on hierarchical partitioning for DOC and conductivity as the response variables (i.e., the stream physical-chemical variables that had the greatest independent contribution for all measures of microbial activity), percent wetland cover on its own explained the most variation in DOC with an independent contribution of 28.7%, while percent forest cover on its own explained just slightly less with an independent contribution of 25.9% (Fig. 5). The independent contributions of percent wetland cover and percent forest only differed by 2.8%, and cumulatively, their independent contributions equaled 54.6%. On its own, road density explained the most variation in conductivity with an independent contribution of 60.5% (Fig. 5). The above independent contributions of percent wetland cover, percent forest cover, and road density were all found to be statistically significant (negative log-likelihood randomization test $P < 0.05$).

DISCUSSION

Our analyses across streams with varying disturbance-induced watershed conditions clearly indicated a link between landscape features and the stream environment, and the stream environment and its leaf-litter microbial communities. Forests and wetlands were identified as providers of essential DOC that stimulated activity of leaf-litter microbial communities in low-order boreal streams. This agrees with previous studies that identified fallen leaf litter as fuel for the aquatic food web (Wallace et al. 1997, Tanentzap et al. 2014), and wetlands rich in vegetation, water, and microbial decomposition as key exporters of DOC (Dillon and Molot 1997). Allochthonous DOC has also been identified as an important food source for stream microbial communities in controlled streamside mesocosms (Kreutzweiser and Capell 2003, Wiegner et al. 2015), and across streams in the United States (Hill et al. 2012). Our study provides further empirical evidence at watershed scales of the key role of forests and their wetlands for providing critical DOC to aquatic microbial communities, and ultimately aquatic food webs.

Increases in the structural complexity of DOC, described using the HIX, were positively correlated

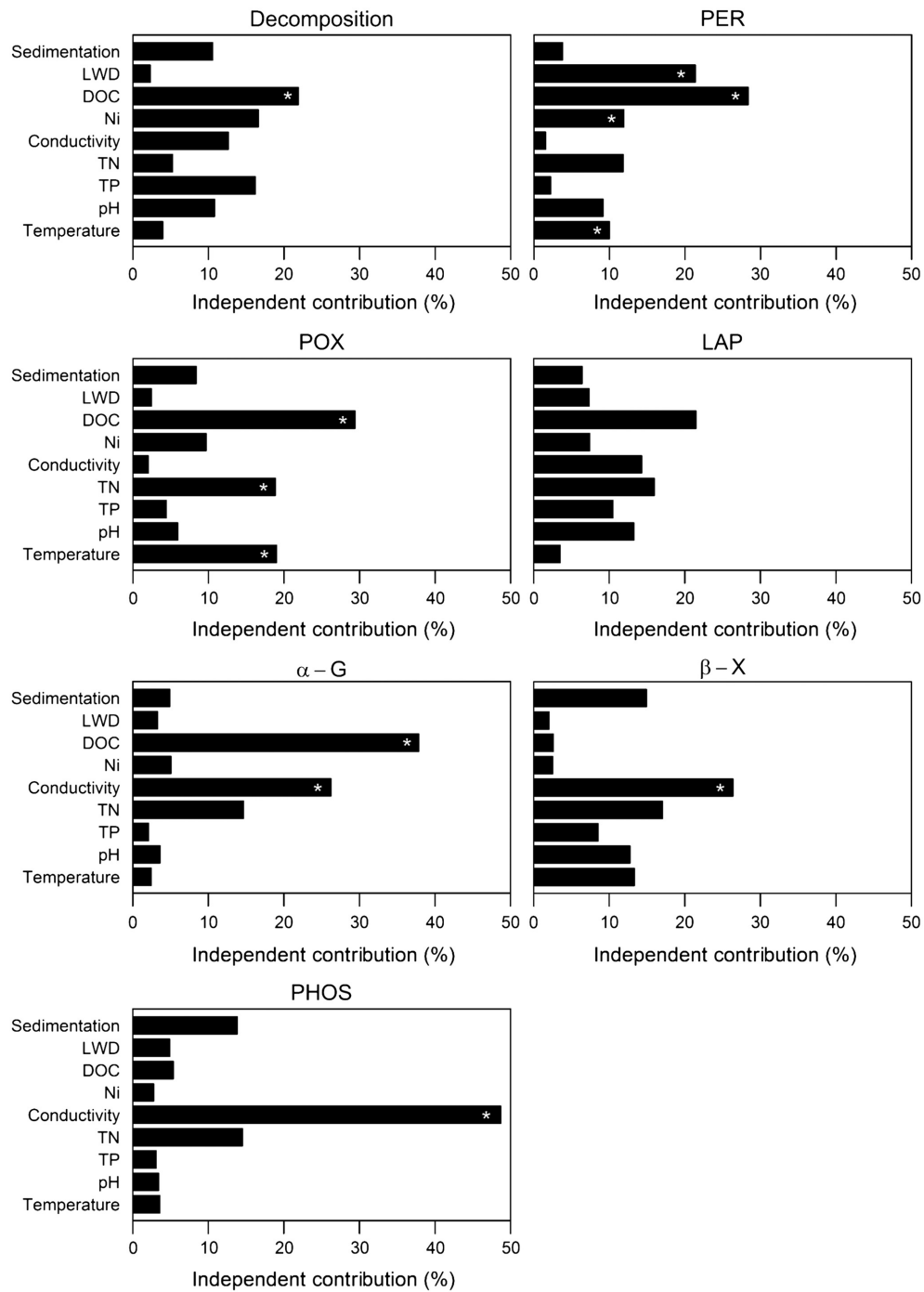


Fig. 3. Independent contribution (%) of each stream explanatory variable for each measure of microbial community activity based on hierarchical partitioning. *Denotes significant independent contribution ($P < 0.05$) based on a negative log-likelihood randomization test ($n = 1000$).

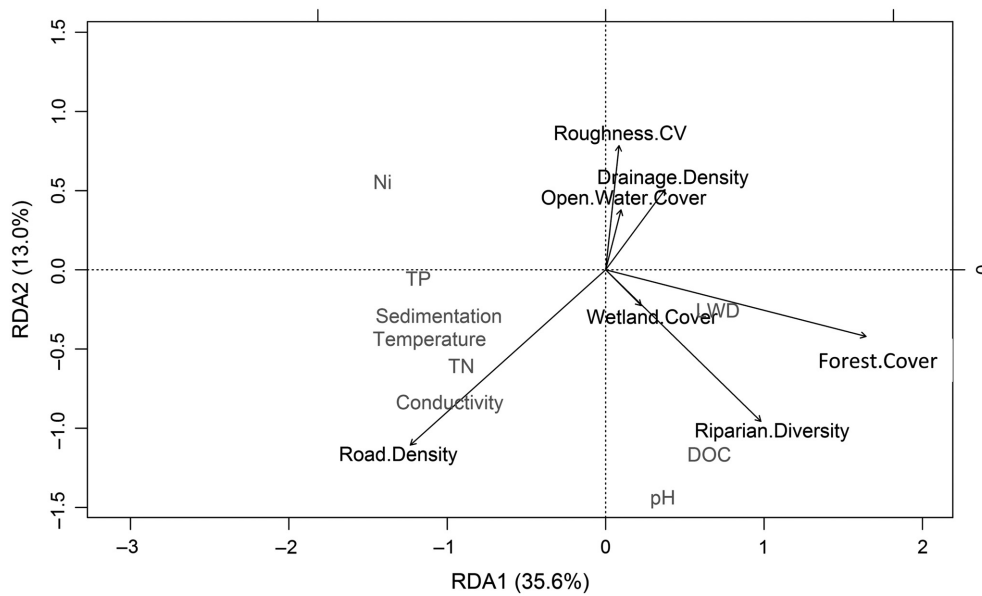


Fig. 4. Redundancy analysis showing associations between stream physical-chemical characteristics and landscape features.

with concentrations of DOC (Pearson’s $r = 0.609$, $P = 0.002$), suggesting that streams with higher DOC concentrations have more terrestrial input and represent a DOC pool that is more humified. The quality of DOC has also been found on its own to influence microbial activity (Hosen et al. 2014). Humified organic matter is typically of terrestrial origin (McKnight et al. 2001), and forest stream ecosystems, like the streams in this study, are known to be heavily dependent on terrestrial inputs (Fisher and Likens 1973). While increases in humic material itself would not likely contribute to increases in the bioavailability of the DOC (Findlay

2003), the higher humification points to higher terrestrial-origin DOC concentrations that were likely the main stimulators of microbial decomposition, α -G, POX, and PER activities. This supports the contention that stream microbial communities are highly adapted and enzymatically queued to the utilization of humified, complex allochthonous DOC (Kreutzweiser and Capell 2003).

Our results suggest that DOC appears to stimulate activity of leaf-litter-decomposing fungal communities more than that of bacterial communities given the positive associations between DOC, fungal biomass, PER and POX activities,

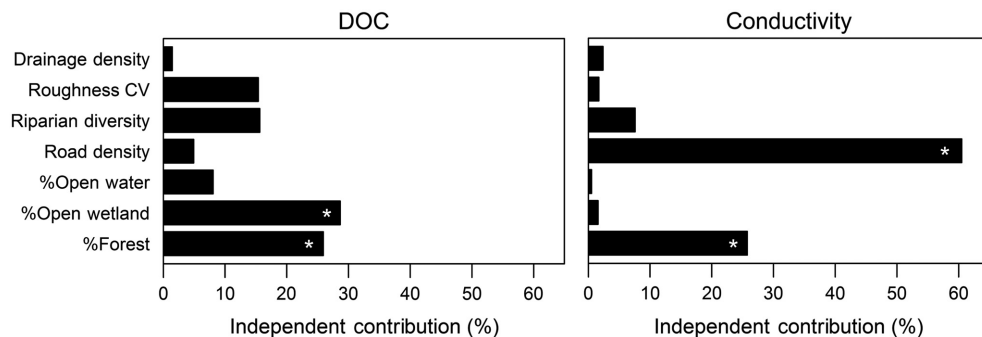


Fig. 5. Independent contribution (%) of each watershed explanatory variable for key stream characteristics based on hierarchical partitioning. *Denotes significant independent contribution ($P < 0.05$) based on a negative log-likelihood randomization test ($n = 1000$).

and microbial leaf-litter decomposition. Although some bacteria can produce PER and POX extracellular enzymes, fungi are the main producers of PER and POX enzymes dedicated to the breakdown of complex carbon molecules such as lignin and cellulose (Kirk and Farrell 1987, Romaní et al. 2006). Fungi are also thought to dominate the initial stages of leaf-litter microbial decomposition (Pascoal and Cássio 2004, Gessner et al. 2007, Duarte et al. 2010), and studies have shown different stoichiometric requirements of fungi (Danger et al. 2016), along with the ability of fungi to supplement leaf-litter decomposition by assimilating required energy and nutrients from stream water (Pastor et al. 2014). Increases in DOC could also be interpreted as a product, instead of a stimulant, of increased fungal biomass and extracellular enzyme activity, and although this study does not prove causation, it should be noted that the microbial communities discussed in this study colonized a standardized substrate during a standardized incubation time and were unlikely on their own to influence the DOC pool in these lotic systems. Additionally, the association between DOC and percent wetland cover further supports the contention that differences in DOC concentrations across streams were a result of DOC transported from the watershed and not a result of in-stream leaf-litter microbial activity.

The α -G also appeared to be stimulated by increased DOC concentration most likely due to intermediate decomposition products from increased PER and POX activities. α -G functions in the degradation of simple starches and is ubiquitously produced by heterotrophic bacterial communities and fungi in aquatic ecosystems (Chróst 1992, Kirchman 2012). On its own, DOC concentration is unlikely to have directly stimulated α -G hydrolase activities given that DOC complexity increased with concentration, and complex allochthonous compounds are less available for hydrolase breakdown. In a study looking at the interaction of fungi and bacteria on leaf litter, bacterial hydrolase enzyme activities increased in the presence of fungi due to fungal POX activities that liberated more readily available compounds from lignin and cellulose (Romaní et al. 2006).

Unlike forests and wetlands, road density was found to negatively impact stream quality and microbial activity. Across the streams in this study,

road density was found to influence water chemistry through increases in conductivity (i.e., ion concentrations), consistent with previous studies on road influences (Trombulak and Frissell 2000). Conductivity in streams was most strongly associated with the main constituents of road dust and salts (i.e., Mg Pearson's $r = 0.892$, $P = <0.001$, and Ca Pearson's $r = 0.879$, $P = <0.001$), suggesting that increased export of road dust and salts to streams may have been responsible. Bacterial biomass and PHOS and β -X hydrolase enzyme activities were influenced by increased conductivities, whereas fungal biomass and PER and POX activities were not influenced, agreeing with a resilience of fungi to contamination (Krauss et al. 2011).

Elevated specific conductance or conductivity appeared to hinder PHOS and β -X activities, while bacterial biomass increased with conductivity. Increases in bacterial biomass coupled with decreases in enzyme activity may be explained by structural changes in the bacterial community. The abundance of Betaproteobacteria was positively associated with PHOS and β -X activities, suggesting that a shift in the bacterial community to a lower abundance of Betaproteobacteria, caused by increased conductivity, could be resulting in lower β -X and PHOS activities. Bacterial community composition has been found to influence hydrolase activities in previous studies. For example, a study by Kirchman et al. (2004) found a positive relationship between an increasing abundance of Betaproteobacteria and PHOS activity in the Hudson River. The association of β -X and PHOS with Betaproteobacteria may also be explained by stoichiometric relationships controlling C:N:P acquisition by hydrolase enzymes, where β -X activity is responding to a P-limitation from decreased PHOS activities (Sinsabaugh and Follstad Shah 2012).

CONCLUSIONS

This study highlights the importance of leaf-litter microbial communities in linkages between terrestrial and aquatic ecosystems where watershed condition and allochthonous DOC export can influence the microbial processing of allochthonous particulate organic matter and subsequent cycling of energy and nutrients. Microbial decomposition and extracellular enzyme activities driven primarily by fungi appeared to be fueled

by DOC supplied by forests and wetlands, while some microbial extracellular enzyme activities appeared to be hindered by structural changes in the bacterial community associated with elevated conductivity from increased road density. Therefore, the restoration and conservation of forests and wetlands in disturbed watersheds appears to be important in providing the supply and transport of DOC to fuel the microbial cycling of energy and nutrients, and thereby advancing the ecological recovery, of aquatic ecosystems. The strategic placement of forest and wetland restoration efforts on the landscape can be optimized by utilizing recent advances in terrain analyses (Creed and Sass 2011, White et al. 2012) to ensure that forest and wetland-derived DOC is enhanced and delivered effectively to receiving waters. Conversely, roads with winter maintenance should be taken into consideration as having a potentially negative impact on the aquatic ecosystem given the potential of elevated conductivities from road sanding and salting to alter the microbial cycling of energy and nutrients. We present leaf-litter microbial communities as a critical link to our understanding and management of the influences of watershed disturbance on aquatic ecosystems.

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