

This is the peer reviewed version of the following article: Swan, C.M. and Sparkman, A. (2023), The role of functional and phylogenetic diversity in riparian tree vegetation on leaf litter breakdown in rivers. *Oikos* e09361. <https://doi.org/10.1111/oik.09361>, which has been published in final form at <https://onlinelibrary.wiley.com/doi/full/10.1111/oik.09361>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.

Access to this work was provided by the University of Maryland, Baltimore County (UMBC) ScholarWorks@UMBC digital repository on the Maryland Shared Open Access (MD-SOAR) platform.

**Please provide feedback**

Please support the ScholarWorks@UMBC repository by emailing [scholarworks-group@umbc.edu](mailto:scholarworks-group@umbc.edu) and telling us what having access to this work means to you and why it's important to you. Thank you.

**The role of functional and phylogenetic diversity in riparian tree vegetation on  
leaf litter breakdown in rivers**

Christopher M. Swan<sup>\*1,2</sup> & April Sparkman<sup>1</sup>

<sup>1</sup>Department of Geography & Environmental Systems

<sup>2</sup>Center for Urban Environmental Research and Education

University of Maryland, Baltimore County

Baltimore, Maryland 21250 USA

\* Corresponding Author:

Christopher Swan

Department of Geography & Environmental Systems

University of Maryland, Baltimore County

Baltimore, Maryland 21250 USA

+1 410 455 3957

Chris.Swan@umbc.edu

## ABSTRACT

While how taxonomic diversity mediates changes in ecosystem function is well-studied, how deeper dimensions of biodiversity drive important processes is understudied. The overarching goal of this work was to determine the role of these dimensions of biodiversity independently and/or interactively in explaining carbon processing in rivers. Here, we explicitly link community structure and subsequent traits of riparian forests to adjacent ecosystem processing of carbon (e.g., leaf litter). This was accomplished by examining how forests are actually structured in addition to experimental manipulations of phylogenetic and functional diversities of riparian forest community inputs of leaf litter to streams. Experimental field manipulations were carried out in three Piedmont headwater streams to answer the following questions: (1) Does *existing* variation in taxonomic, functional and phylogenetic diversity of riparian communities differentially drive decomposition in rivers? And (2) *Independent* of taxonomic diversity, how does functional and phylogenetic diversity of leaf litter assemblages influence rates of decomposition in rivers? We observed significant interspecific variation in breakdown among 30 riparian tree species, in addition to significant relationships between breakdown rate and important foliar tissue chemistries. Breakdown of mixtures that reflected the composition of the riparian species composition did not vary with functional nor phylogenetic diversity, but breakdown of litter mixtures was higher than that of single species. In a separate study, when manipulated independently, functional and phylogenetic diversity were positively related to breakdown, and explained similar degrees of variation. These results are important to understand in light of deepening knowledge of the role different dimensions of biodiversity take in explaining ecosystem function.

KEYWORDS: biodiversity, carbon processing, ecosystem function, functional diversity, leaf litter, phylogenetic diversity, riparian

## INTRODUCTION

Biodiversity is the foundation for nearly all ecosystem services (Chapin et al. 2000, Hooper et al. 2005). How species loss confers changes to the rates of ecosystem functions/services has been the focus of intense research for decades. Ecologists are increasingly embracing, though, different dimensions of biodiversity in their evaluation of such constraints (Cadotte et al. 2009, Cavender-Bares et al. 2009, Flynn et al. 2011, Ebeling et al. 2014). For example, loss of phylogenetic diversity and/or distinctiveness in a community and subsequent effects on ecosystem processes provides a different and more nuanced interpretation than loss of taxonomic diversity alone (Webb et al. 2002, Cadotte 2013). However, such effects need to be largely interpreted through the lens of functional trait composition and/or diversity of a community since it is likely species traits explain patterns of ecosystem function better than taxonomic richness or phylogenetic diversity alone (Lecerf et al. 2011, Flynn et al. 2011).

In temperate ecosystems, the majority of terrestrial primary production enters the detrital pool (Cebrian 1999). Breakdown and decomposition of senesced carbon and release of nutrients are key ecosystem processes responsible for soil formation and the transfer of energy to higher trophic levels in both aquatic and terrestrial environments (Gessner et al. 2010). As such, decades of research have revealed much about what abiotic and biotic factors mediate rates of decomposition in both terrestrial and adjacent aquatic ecosystems (e.g., Webster and Benfield 1986, Lecerf et al. 2007, Follstad Shah et al. 2017, Tiegs et al. 2019). In general, decomposition rates are explained by climate/environment, substrate quality, and metazoan feeding (Webster and Benfield 1986, Boyero et al. 2016). In terms of substrate quality, nutrients (e.g., N, P) are known to increase breakdown rate, while carbon-rich and secondary compounds inhibit the rate of breakdown (Webster and Benfield 1986, Ostrofsky 1993, 1997). There exists high interspecific variation in foliar chemistry among plant species (Ostrofsky 1993, 1997). As such, there has been focus on how species-specific rates of breakdown vary, in addition to how loss of riparian forest biodiversity has implications for carbon processing in both terrestrial and adjacent aquatic ecosystems (Gessner et al. 2010, Boyero et al. 2021).

Since (Gessner et al. 2010), there has been relatively fewer studies focusing on how loss and/or shifts in forest species composition confers changes to decomposition rates in soils and streams. Decades of work leading up to this paper demonstrated that the effect traits of leaves described above (i.e., N, lignin content) do explain variation in the decomposition of detritus (Suberkropp et al. 1976, Hladysz et al. 2009). However, the degree to which shared evolutionary history helps explain these patterns is largely unknown, yet important to understand (LeRoy et al. 2020). This is because species loss can translate into loss of such traits. Species, however, exhibit different degrees of phylogenetic distinctiveness. If traits show phylogenetic signal, that is more closely related species are functionally more similar, then managing species for phylogenetic distinctiveness may be important to consider if functionally important species are of concern. However, if traits do not show phylogenetic signal, then the degree of shared evolutionary history is likely uninformative in helping to understand interspecific variation in carbon processing of senesced leaf litter.

There is evidence to support the hypothesis that phylogenetic diversity is important to both changes in ecosystem processes and species functional role in the environment (Cadotte et al. 2009, Cavender-Bares et al. 2009, Cadotte 2013). But, phylogenetic diversity (PD), which accounts for evolutionary distinctiveness between species, is understudied in the context of carbon processing. Recent works suggest that PD can be used as a proxy for measures of functional diversity (FD), contingent on the assumption that evolutionary diversification has caused trait diversification and closely related species should be more ecologically similar (Srivastava et al. 2009, Ackerly 2009). If this is true, then ecosystem function should increase with PD through a complementarity effect, for which highly diverse assemblages efficiently use resources through positive interactions or niche partitioning (Flynn et al. 2011, Srivastava et al. 2012). In many cases, high quality trait data and knowledge of all traits influencing any one ecosystem function can be lacking, therefore PD could be a valuable proxy for FD (Srivastava et al. 2012, Boyero et al. 2021).

Given evidence that both functional and phylogenetic diversity are acceptable predictors for a variety of ecosystem processes (Cadotte et al. 2009, Flynn et al. 2011), there is less of an understanding as to how

functional effect trait composition and phylogenetic composition of exhibited by leaf litter derived from riparian forest communities can drive carbon processing in adjacent streams (but see López-Rojo et al. 2020). If phylogenetic diversification has caused diversification in traits relevant to the decomposition process, then a phylogenetically distinct species could be crucial in driving carbon processing in rivers. Most empirical studies that have examined the relationship between FD or PD and ecosystem function have experimentally created communities (e.g., Connolly et al. 2011, Cadotte 2013). Few studies have explored *existing* community structure and its effect on various ecosystem processes. In this context, we offer that riparian tree communities and the underlying mechanisms that drive riparian forest community assembly could indirectly, but significantly, influence carbon processing in streams via diverse inputs of leaf litter of different foliar chemistries. If environmental filtering or competitive exclusion are working to structure riparian forest communities, there is the potential for both phylogenetically and functionally overdispersed and underdispersed community structures to occur (HilleRisLambers et al. 2012). These types of assembly outcomes have the potential to alter community “effect” trait composition (Cavender-Bares et al. 2009, Mayfield and Levine 2010) and subsequently decomposition of leaf litter in adjacent waterways (López-Rojo et al. 2020).

There are several drawbacks when one attempts to use both functional and phylogenetic diversity as predictors for ecosystem function. The first is that if traits are conserved along the phylogeny, i.e. they show phylogenetic signal, trait patterns align with phylogenetic patterns. This is not always the case, as many species exhibit substantial intraspecific trait variation (Lecerf and Chauvet 2008) or there are evolutionary processes at work such as convergent evolution that can cause species to independently evolve similar traits despite distant phylogenetic relationships. A consequence is that ambient functional and phylogenetic diversities are significantly correlated with one another. In this case, experimental manipulations are necessary in order to tease apart how each of these dimensions independently influences ecosystem function.

We performed two studies. The first examined existing riparian community structure, while the second was an experimental manipulation of FD and PD to explore their independent and interactive roles in

driving decomposition rates. We assembled a phylogenetic tree for the riparian species occurring in the Mid-Atlantic Region of the U.S. and estimated a comprehensive suite of relevant foliar functional traits known to drive breakdown rate in aquatic systems. Armed with this information, we asked the following questions: 1) What are the patterns in functional traits and decomposition for a large suite of riparian species? 2) Does functional or phylogenetic diversity of naturally occurring riparian communities drive rates of decomposition across multiple streams? and 3) by experimentally creating treatments with phylogenetic and functional diversities *uncorrelated* and holding species richness *constant*, does functional and phylogenetic diversity explain variation in breakdown rates? We predicted that, as other studies have shown, mixtures of leaf species leaf litter would breakdown faster than single species. Further, patterns of decomposition would be related with each of the measured functional traits, where carbon processing rates would increase with increases in N and P, and decrease with increasing total phenolics, toughness, and C:N content. In naturally occurring riparian communities, we expected that breakdown rates will be positively correlated with both FD and PD. When FD and PD are independently manipulated, we predicted that FD and PD will both explain variation in litter processing rates, with more variation explained by PD. PD is hypothesized to take into account unmeasured yet important traits for function and, therefore, PD will explain more variation than a dimension that takes into account a limited number of traits (Boyero et al. 2021).

## **METHODS**

### *Study Site*

This work was conducted in three, spring-fed headwater streams draining into the Patapsco River, located in Howard County, Maryland, USA. The three headwater streams were denoted as Cascade (39.232, -76.758), Patapsco (39.232, -76.743), and Soapstone (39.244, -76.728) based on location adjacent to trails as part of Patapsco Valley State Park. The Piedmont watershed is covered by >99% forest and streams exhibit a typical riffle run geomorphology, dominated by small rocks and gravel. The riparian communities are dominated by American beech, White oak, Red maple and American sycamore (Swan et al. 2009a). The study reach for each of the streams was approximately 50-100 m, depending on local geomorphological conditions. At Cascade, the average channel width is ~105 cm with an average

water depth of 5.97 cm and base flow discharge of  $0.17 \text{ m}^3 \text{ s}^{-1}$ . Patapsco had an average channel width of 146 cm with an average depth of 9.47 cm and base flow discharge of  $0.15 \text{ m}^3 \text{ s}^{-1}$ . At Soapstone, the average channel width is 94 cm with an average water depth of 8.8 cm and base flow discharge of  $0.2 \text{ m}^3 \text{ s}^{-1}$ . All streams had average water temperatures of  $10.2^\circ\text{C}$  during the study period.

## *Study 1: The Role of Existing Riparian Taxonomic, Functional and Phylogenetic Diversity on Litter*

### *Breakdown*

#### *Litter Composition*

Riparian forest composition of streams in the Mid-Atlantic region was determined using data from the US Forest Inventory and Analysis dataset (FIA). This dataset comprises species composition of regional riparian plots and site-specific foliar biomass. The riparian plots and species list for each plot were used to identify the majority of deciduous riparian species in the region. Locally accessible individuals of each species were then identified from this list and leaves collected from each tree species directly following abscission during the Fall of 2013. A total of 30 tree species were included in this study (Table 1). After collection, leaves were taken back to the lab where they were stored and air dried in paper bags. Leaves were mixed and rotated daily within bags until completely air-dried.

In order to examine how existing taxonomic, functional, and phylogenetic diversity in riparian tree communities influences carbon processing in streams, tree communities located adjacent to water were identified and extracted from the FIA dataset. By using site-specific foliar biomass as an estimate of relative abundance of leaf litter biomass that could potentially enter an adjacent stream and functional “effect” trait data, functional and phylogenetic diversities were calculated for existing riparian assemblages.

#### *Functional Traits - Analysis of Foliar Chemistry*

Foliar chemistry was taken as the functional traits for this study (Lecerf et al. 2011) and were determined on ground, senesced leaf litter from each species (Table 1). Leaves were ground with a mill and stored in



plastic vials labeled 1-30, each number representing a species to avoid bias during analysis. Chemical analysis of samples was performed at the University of Coimbra, Coimbra, Portugal.

C:N and %N were determined using EA-IRMS (Elemental analyzer isotope ratio mass spectrometry). The analysis involves combustion or thermal conversion of sample material in the elemental analyzer, which is then introduced to the ion source of the mass spectrometer. %P was analyzed using a basic digestion so that all phosphorous in ground leaf material was converted to soluble reactive phosphorous (SRP). Afterwards basic SRP technique was applied and samples were read at 825 nm on a spectrophotometer (Shimadzu Biospec-Mini). All analyses were done in triplicate and averaged for mean %P. Total phenolics were determined by digesting leaf material in 50% acetone. After digestion, a Folin Denis solution was added to the digested material and read on a spectrophotometer at 725 nm following (Bärlocher et al. 2020).

Toughness, which was used as a proxy for structural integrity imparted by fiber, such as lignin, cellulose and hemicellulose concentrations (Graça and Zimmer 2020), was determined using a penetrometer. Three leaves per species were placed one at a time between two marble slabs and a needle (0.79 mm diameter) placed through those slabs onto the leaf material. A beaker was placed on the top of that needle and the average mass (g) of water needed to break the leaf was taken as toughness. Analysis here was done in triplicate per leaf, where toughness was determined by averaging 9 replicates per species (Graça and Zimmer 2020).

### *Phylogenetic Analysis*

We used the command-line program phyloGenerator (Pearse and Purvis 2013) to create the phylogenetic tree. This method is based on the BioPython framework (Talevich et al. 2012) and automates the retrieval of sequence information from Genbank (Benson et al. 2013), as well as alignment and phylogenetic tree estimation. We constructed this tree based on the commonly sequenced genes *rbcl*, *matK* and *trnL* genes. The trimmed sequences were aligned using MAFFT (Katoh and Standley 2013) after being

visually examined for misalignment using Clustal X (Larkin et al. 2007). We generated a maximum-likelihood tree using RaxML (Stamatakis 2014), using 1000 accelerated bootstrap replicates (Stamatakis et al. 2008), and then rate-smoothed the tree to be ultrametric using the PATHd8 algorithm, with *Asimina triloba* set as an outgroup (Britton et al. 2007, Fig.1).

#### *Calculating Functional and Phylogenetic Diversities*

Several indices for functional diversity have been introduced and incorporated into ecological research (Petchey and Gaston 2002, Vill  ger et al. 2008, Laliberte and Legendre 2010). For this analysis, functional diversity was calculated as functional dispersion on C:N, %N, %P, Toughness and Phenolics (Table 2). This measure weights the distribution of functional traits in a “community” (here, litter species) by species proportions (FDis; (Laliberte and Legendre 2010). All indices were calculated using the “FD” and “picante” packages in R (Vill  ger et al. 2015).

Phylogenetic diversity can also be calculate in several different ways, however here we chose to use mean phylogenetic distance (MPD; Vellend et al. 2007, Cadotte et al. 2010). MPD is most widely used and is calculated as the mean phylogenetic distance between each pair of species in the dataset to take into account deep evolutionary relationships. This distance-based measure is then compared to a null model, which is created from drawing random samples from the pool of species in the phylogeny. These comparisons allow for interpretation as to whether the community exhibits high or low phylogenetic diversity (Cadotte et al. 2010). We chose to use MPD over other measures as it more evenly takes into account differences across the entire community e.g., (Johnson et al. 2015) (Table 2).

#### *Study Design*

Once functional and phylogenetic diversities were calculated, we determined species richness across all plots identified in the FIA dataset. All riparian communities were then plotted in a functional vs phylogenetic “diversity” space. A subset of communities were chosen based on observed gradients in functional and phylogenetic diversity, where combinations of high/low of each dimension were identified. A total of 16 communities were selected, maintaining species richness as consistently as possible,

252 ranging from 3-6 species. This is reasonable given the limited literature on how many leaf species co-  
253 occur together as an assemblage on the streambed (Swan and Palmer 2004). Litter mixture treatments  
254 were created reflecting the taxonomic structure and relative species abundance, taken as foliar biomass,  
255 of each riparian plot, as it is documented that litter species evenness is important to how diversity affects  
256 breakdown rates in streams (Swan et al. 2009a).

257  
258 Single species treatments were created for all 30 species (Figs 1 & 2). Treatments were created using  
259 mesh litter bags measuring approximately 20 cm x 10 cm with a mesh size of 7 x 11 mm (Swan and  
260 Palmer 2004, Swan et al. 2009b, 2009a). Each single and each of the 16 mixture treatments were  
261 replicated 4 times per stream, for a sample size of n=12 for single and mixed species treatments across  
262 three streams (Cascade, Patapsco, Soapstone). Each mesh bag comprised an average of 5 g per litter  
263 bag and in several cases, larger species were cut to size when necessary. A loss estimate bag was  
264 created for each treatment, also containing approximately 5 g to estimate how much mass loss was due  
265 to handling in route to the streams. A total of 620 bags were deployed across all treatments and streams.

266  
267 Litter bags were created and stored by replicate for approximately one week, after which they were taken  
268 to the field. Four replicates per treatment were then attached to metal wires and nailed to the streambed  
269 in riffles, following (Swan et al. 2009a). The replicates were placed at random, using a block design  
270 whereby single replicates were grouped together in the stream to account for any unknown spatial  
271 gradient imposing constraints on mass loss, and were equally spaced along the wire. Leaf treatments  
272 were deployed in mid-March 2014 then left for ~40 days and retrieved in late April 2014. During this 40-  
273 day period, leaf packs were monitored weekly and in the event of large rain events some leaf packs were  
274 removed from burial. Upon final removal, the bags were clipped from the wire and placed directly into a  
275 plastic bag and kept in a cooler for transport to the lab for processing. The coolers were immediately  
276 taken back to the lab and leaf packs were stored in the refrigerator. Leaf packs were processed within 24  
277 hours, rinsing leaves of sediments then leaving to air dry for one week. Each sample was then placed in  
278 the drying oven for 48 hours and ash-free dry mass determined by combusting at 550° C for 2 hours  
279 (e.g.,(Swan et al. 2009a).

## *Study 2: Independent Manipulation of Functional and Phylogenetic Diversities*

To investigate the independent roles of functional and phylogenetic diversities, we again utilized the Forest Inventory and Analysis (FIA) dataset to determine dominant riparian tree species for the Piedmont region. Using the total relative abundance across all plots in this dataset, a subset of species were chosen that are likely to contribute significant biomass of senesced leaf litter to headwaters. We randomly assembled 16 communities with a species richness of 4, which was the average number of riparian tree species observed per site in the FIA. Functional and phylogenetic diversity were then subsequently calculated for  $n=1000$  randomly assembled communities. This approach eliminated confounding species richness with each of these dimensions. Communities were constructed in a design of high/low functional diversity (FD) and high/low phylogenetic diversity (PD) based on their position in diversity space. Litter mixtures with these varying levels of FD and PD were created using leaf litter from locally occurring species across these communities collected in the Fall of 2014.

Following the same methods above, each assembled "community" was replicated 4 times. Litter bags comprised 5 g of leaf litter, with equal proportions of each of the four species allocated to mixtures. Litter bags were also created for all single species. Litter bags were placed across the same three streams using the same methods as above, and sampled/processed similarly to quantify breakdown rate. Leaves were deployed in mid-March 2015 and were retrieved in late April 2015 for a total of 42 days. A total of 420 litter bags were deployed across all treatments and streams.

## *Statistical Analysis*

Breakdown rate was taken as the difference between mass loss initially and after exposure to the stream environment, then divided by number of days in the stream (Webster and Benfield 1986). To address how breakdown rates differed across species, a one-way analysis of variance was performed to first determine differences in the rate of breakdown among species for 2014. Stream location was modeled as a block to better isolate the effect of species on breakdown rate.

We examined how individual functional traits - % N, % P, Phenolics, Toughness and C:N content – explained variation in single species breakdown separately in 2014 and 2015. Using a general linear modeling approach, we accounted for stream site as an intercept effect in models separately for each trait explaining variation in breakdown. We report the slope of the relationship, significance level and adjusted  $r^2$ .

Similarly, for the 2014 study, to understand the relationship between decomposition rates and existing taxonomic, functional, and phylogenetic diversity, regression analyses were performed with a general linear model, with stream modeled as an intercept effect, then FD and PD included as explanatory variables. As species richness was held constant across all mixtures in 2015, the GLM approach, while similar to that used to analyze the 2014 results, only included FD and PD (in addition to stream site as an intercept). As both FD and PD were highly correlated with Shannon diversity, a separate simple linear regression was performed to aid in understanding the results. To assess the effect of litter mixing on 2014 breakdown rates, observed breakdown of mixed litter was averaged across replicates within stream and subtracted from the predicted breakdown based on the weighted average of the single species breakdown rates in those same streams. Similarly, for 2015, observed breakdown of mixed litter averaged across replicates within stream was subtracted from the predicted breakdown calculated based on the average of the four single species breakdown rates in those same streams. A separate test of non-additivity for each year was assessed with a one-sample t-test. Our prediction was that mixtures overall will exhibit higher rates of breakdown than expected based on single species estimates.

All analyses were conducted in R version 4.0.2 (R Core Team 2020). All of the data were examined to determine that the assumptions of normality and equal variances were met (Shapiro-Wilks Test, Levene's test), and statistical significance assessed at  $\alpha=0.05$ .

## RESULTS

### *Study 1: The Role of Existing Riparian Taxonomic, Functional and Phylogenetic Diversity on Litter Breakdown*

In the largest study of its kind in stream ecosystems, in terms of the sheer number of leaf species studied in concert in the same system (see (Ostrofsky 1993, 1997) for lentic examples) we observed significant variation in breakdown among litter species ( $F_{29,309} = 11.8$   $P < 0.001$ , Fig. 2). Further, breakdown rate was positively related to both percent phosphorus and nitrogen content, while negatively related to both phenolic content and tissue toughness (Table 3). These relationships exhibited substantial variability, all with  $r^2$  less than 0.30 (Table 3).

A general linear model relating breakdown to PD and FD was performed with stream locations modeled as intercept effects, and no interactions were found to be significant (Table 4). Neither dimension of biodiversity explained significant variation in breakdown (Fig. 3a-b). Furthermore, both were significantly correlated with Shannon diversity (PD:  $r = 0.77$ ,  $t_{43} = 7.86$ ,  $P < 0.001$  ; FD:  $r = 0.57$ ,  $t_{43} = 4.56$ ,  $P < 0.001$ ), limiting interpretation of the relationships with FD and PD (Table 2, see Discussion). However, mixing litter resulted in significant deviations from expected breakdown based on weighted averages of single species estimated ( $t_{df=47} = 2.68$ ,  $P < 0.01$ , Fig. 3c). More than 60% of the mixtures under study broke down faster than expected, suggested species diversity was important more in accelerating ecosystem function than impeding it (e.g., Lecerf et al. 2011, Swan et al. 2009a, Swan 2011).

### *Study 2: Independent Manipulation of Functional and Phylogenetic Diversities*

Since Shannon diversity was significantly correlated with both FD and PD for natural patterns of leaf litter composition, we experimentally created treatments with phylogenetic and functional diversities *uncorrelated* and held species richness *constant*, to learn if functional and phylogenetic diversity explain variation in breakdown rates. The general linear model relating breakdown to stream site (intercept effect) and FD and PD was highly significant for all sources of variation (Table 4). Both dimensions of biodiversity were significantly and positively related to breakdown (Fig 4a-b). However, unlike in 2014,

mixing of litter species was not found to be important to driving any non-additivity in ecosystem function  
( $t_{df=47} = 0.20$ ,  $P=0.422$ , Fig. 4c).

## DISCUSSION

We report the results of two studies examining the afterlife effects of functional and phylogenetic diversity on leaf litter breakdown in three headwater streams. Consistent with our predictions, we found significant interspecific variation in breakdown rate among the 30 riparian species studied here. In addition, breakdown varied significantly and predictably with key foliar chemistries, namely N, P, total phenolics and tissue toughness. Breakdown of mixtures that reflected the composition of riparian species as reported in the FIA database did not vary with functional diversity nor phylogenetic diversity as calculated here, but was explained more by site effects. However, when functional and phylogenetic diversity were manipulated independent of one another in mixtures of four species from the same species pool, breakdown was positively related to both measures of biodiversity, and explained very similar amounts of variation (taken as  $r^2$ ). We offer that these results are important to understand in light of deepening knowledge of the role different dimensions of biodiversity take in explaining ecosystem function.

In naturally occurring riparian communities, we expected that breakdown rates would be positively correlated with both functional and phylogenetic diversities. This was not the case. Here, stream site explained significant variation in breakdown, but no additional variation was explained by these measures of biodiversity. This result is blurred, though, as we observed a significant non-additivity, or species diversity effects, of mixing on breakdown. We did observe that species richness was significantly correlated with FD and PD. The lack of any relationship with breakdown and these two dimensions of biodiversity may likely have been to the fact that we maintained natural species evenness in the litter bags.

We predicted that when functional diversity and phylogenetic diversity were independently manipulated, both would increase breakdown rates, with phylogenetic diversity explaining more variation than functional diversity. This was because this dimension of diversity takes into account a limited number of traits (Boyer

et al. 2021). While both dimensions were significantly and positively related to breakdown, both, surprisingly, explained nearly the same amount of variation (Table 2). One interpretation is that our choice of traits was comprehensive, or at least correlated with traits that were those explained by the phylogenetic relationships estimated here (but see below).

Phylogenetic diversity was hypothesized to take into account unmeasured yet important traits for function and would, therefore, explain more variation than functional diversity (Srivastava et al. 2012). This prediction did not hold as both dimensions explained roughly the same amount of variation in breakdown. Nevertheless, the significance of the positive relationship between breakdown and phylogenetic diversity suggests unmeasured traits that are associated with distinguishing species are important to explaining breakdown. This is because, owing to our experimental design, we held other factors constant that are known to influence breakdown across all litter combinations, namely environmental factors like flow, temperature and water chemistry, and micro- and macro-consumers. Unmeasured traits might include detailed components that explain toughness, such as cellulose, hemicellulose and lignin content (Webster and Benfield 1986, Ostrofsky 1997). Furthermore, while total phenolics was a significant predictor of breakdown (Table 3), it could also be that different phenolic chemistries play stronger or weaker roles in regulating this process. Phylogenetic diversity of riparian tree assemblages seems an important measure with respect to how it relates to breakdown, and further suggest other traits than those here are important to the breakdown process. For example, fiber components, such as lignin that was not isolated here, is known to be important to breakdown, especially to leaf degrading consumers (e.g., fungi, invertebrates; Hladysz et al. 2009)

Leaf-chewing consumers are known to be important regulators on the breakdown process, especially in small streams under study here (Sponseller and Benfield 2001). Prior work in this system has revealed a diverse “shredder” community, namely *Allocapnia* sp, *Amphinemoura* sp, *Anchytarsus* sp, *Gammarus pulex*, *Hydrochus* sp., *Nemoura* sp, *Pycnopsyche gentilis* and *Tipula* sp (Swan 2011, personal observation). Furthermore, it is known that shredders respond to diverse leaf litter resources in terms of feeding rates, both preferentially and in a compensatory fashion, and can exacerbate the effect of leaf



litter diversity on the breakdown process (Swan and Palmer 2006a, 2006b, Sanpera-Calbet et al. 2009). An interesting, yet herculean, study would be to double the size of what is reported here and cross the treatments with a coarse versus fine mesh size litter bag manipulation to test the effect of excluding consumers.

One of the most common approaches to restoring stream habitat is riparian reforestation (Sweeney and Czapka 2004). Trees provide shade, bank stabilization, habitat, and inputs of energy as leaf litter to adjacent waterways (Sweeney 1993). Thus, tree plantings offer a large benefits relative to implementation effort. Practitioners often choose different species assemblages based on their life history traits, e.g., growth rate, in their planting designs (Sweeney and Czapka 2004), or to address species loss. For example, in this region, ashes are under threat and thus the implications for riparian structure and function are of direct applied concern (i.e. Emerald Ash Borer invasion; (Jennings et al. 2016)). When litter inputs as energy sources for instream food webs is cited (e.g., (Sweeney 1993, Jones and Swan 2016) foliar chemistry has been considered superficially, but not in detail. The consequences in the in stream potentially include shifts in organic matter processing rates, potential alterations to the flow of resources to various food web compartments, and the residence time of carbon. This research reports results directly tying riparian community structure to both existing patterns of phylogenetic and functional diversity, and potential planting schemes that represent different diversities of these dimensions to instream processing.

#### ACKNOWLEDGEMENTS

The authors thank Dorothy Borowy, Sarah Hutchinson, Anna Johnson, William Swan, Francisco Valente-Neto, Nicole Voelker and Charles Wahl for help with both the laboratory and field work.

#### REFERENCES

Ackerly, D. 2009. Conservatism and diversification of plant functional traits: Evolutionary rates versus phylogenetic signal. *Proceedings of the National Academy of Sciences of the United States of America* 106:19699–19706.

448 Bärlocher, F., M. O. Gessner, and M. A. S. Graça, editors. 2020. Methods to Study Litter Decomposition:  
 449 A Practical Guide. Springer, Cham.

450 Benson, D. A., M. Cavanaugh, K. Clark, I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and E. W. Sayers.  
 451 2013. GenBank. Nucleic Acids Research 41.

452 Boyero, L., R. G. Pearson, C. Hui, M. O. Gessner, J. Pérez, M. A. Alexandrou, M. A. S. Graça, B. J.  
 453 Cardinale, R. J. Albariño, M. Arunachalam, L. A. Barmuta, A. J. Boulton, A. Bruder, M. Callisto, E.  
 454 Chauvet, R. G. Death, D. Dudgeon, A. C. Encalada, V. Ferreira, R. Figueroa, A. S. Flecker, J. F.  
 455 Gonçalves, J. Helson, T. Iwata, T. Jingtut, J. Mathooko, C. Mathuriau, C. M'Erimba, M. S.  
 456 Moretti, C. M. Pringle, A. Ramírez, L. Ratnarajah, J. Rincon, and C. M. Yule. 2016. Biotic and  
 457 abiotic variables influencing plant litter breakdown in streams: A global study. Proceedings of the  
 458 Royal Society B: Biological Sciences 283:20152610–20152664.

459 Boyero, L., J. Pérez, N. López-Rojo, A. M. Tonin, F. Correa-Araneda, R. G. Pearson, J. Bosch, R. J.  
 460 Albariño, S. Anbalagan, L. A. Barmuta, L. Beesley, F. J. Burdon, A. Caliman, M. Callisto, I. C.  
 461 Campbell, B. J. Cardinale, J. J. Casas, A. M. Chará-Serna, S. Ciapała, E. Chauvet, C. Colón-  
 462 Gaud, A. Cornejo, A. M. Davis, M. Degebrodt, E. S. Dias, M. E. Díaz, M. M. Douglas, A. Elozegi,  
 463 A. C. Encalada, E. de Eyto, R. Figueroa, A. S. Flecker, T. Fleituch, A. Frainer, J. S. França, E. A.  
 464 García, G. García, P. García, M. O. Gessner, P. S. Giller, J. E. Gómez, S. Gómez, J. F.  
 465 Gonçalves Jr, M. A. S. Graça, R. O. Hall Jr, N. Hamada, L. U. Hepp, C. Hui, D. Imazawa, T.  
 466 Iwata, E. S. A. Junior, S. Kariuki, A. Landeira-Dabarca, M. Leal, K. Lehosmaa, C. M'Erimba, R.  
 467 Marchant, R. T. Martins, F. O. Masese, M. Camden, B. G. McKie, A. O. Medeiros, J. A.  
 468 Middleton, T. Muotka, J. N. Negishi, J. Pozo, A. Ramírez, R. S. Rezende, J. S. Richardson, J.  
 469 Rincón, J. Rubio-Ríos, C. Serrano, A. R. Shaffer, F. Sheldon, C. M. Swan, N. S. D. Tenkiano, S.  
 470 D. Tiegs, J. R. Tolod, M. Vernasky, A. Watson, M. J. Yegon, and C. M. Yule. 2021. Latitude  
 471 dictates plant diversity effects on instream decomposition. Science Advances 7.

472 Britton, T., C. L. Anderson, D. Jacquet, S. Lundqvist, and K. Bremer. 2007. Estimating divergence times  
 473 in large phylogenetic trees. Systematic Biology 56:741–752.

474 Cadotte, M. W. 2013. Experimental evidence that evolutionarily diverse assemblages result in higher  
 475 productivity. *Proceedings of the National Academy of Sciences of the United States of America*  
 476 110:8996–9000.

477 Cadotte, M. W., J. Cavender-Bares, D. Tilman, and T. H. Oakley. 2009. Using phylogenetic, functional  
 478 and trait diversity to understand patterns of plant community productivity. *PloS One* 4.

479 Cadotte, M. W., T. Jonathan Davies, J. Regetz, S. W. Kembel, E. Cleland, and T. H. Oakley. 2010.  
 480 Phylogenetic diversity metrics for ecological communities: integrating species richness,  
 481 abundance and evolutionary history. *Ecology Letters* 13:96–105.

482 Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community  
 483 ecology and phylogenetic biology. *Ecology Letters* 12:693–715.

484 Cebrian, J. 1999. Patterns in the fate of production in plant communities. *The American Naturalist*  
 485 154:449–468.

486 Chapin, F. S., 3rd, E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U.  
 487 Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Díaz. 2000. Consequences of  
 488 changing biodiversity. *Nature* 405:234–242.

489 Connolly, J., M. W. Cadotte, C. Brophy, D. Álne, J. Finn, L. Kirwan, C. Roscher, and A. Weigelt. 2011.  
 490 Phylogenetically diverse grasslands are associated with pairwise interspecific processes that  
 491 increase biomass. *Ecology* 92:1385–1392.

492 Ebeling, A., S. Pompe, J. Baade, N. Eisenhauer, H. Hillebrand, R. Proulx, C. Roscher, B. Schmid, C.  
 493 Wirth, and W. W. Weisser. 2014. A trait-based experimental approach to understand the  
 494 mechanisms underlying biodiversity-ecosystem functioning relationships. *Basic and Applied*  
 495 *Ecology* 15:229–240.

496 Flynn, D. F. B., N. Mirotchnick, M. Jain, M. I. Palmer, and S. Naeem. 2011. Functional and phylogenetic  
 497 diversity as predictors of biodiversity- Ecosystem-function relationships. *Ecology* 92:1573–1581.

498 Follstad Shah, J. J., J. S. Kominoski, M. Ardón, W. K. Dodds, M. O. Gessner, N. A. Griffiths, C. P.  
 499 Hawkins, S. L. Johnson, A. Lecerf, C. J. LeRoy, D. W. P. Manning, A. D. Rosemond, R. L.  
 500 Sinsabaugh, C. M. Swan, J. R. Webster, and L. H. Zeglin. 2017. Global synthesis of the

501 temperature sensitivity of leaf litter breakdown in streams and rivers. *Global Change Biology*  
502 23:3064–3075.

503 Gessner, M. O., C. M. Swan, C. K. Dang, B. G. McKie, R. D. Bardgett, D. H. Wall, and S. Hättenschwiler.  
504 2010. Diversity meets decomposition. *Trends in Ecology & Evolution* 25:372–380.

505 Graça, M. A. S., and M. Zimmer. 2020. Physical Litter Properties: Leaf Toughness and Tensile Strength.  
506 Pages 187–193 in F. Bärlocher, M. O. Gessner, and M. A. S. Graça, editors. *Methods to Study*  
507 *Litter Decomposition: A Practical Guide*. Springer International Publishing, Cham.

508 HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking  
509 community assembly through the lens of coexistence theory. *Annual Review of Ecology,*  
510 *Evolution, and Systematics* 43:227–248.

511 Hladysz, S., M. O. Gessner, P. S. Giller, J. Pozo, and G. Woodward. 2009. Resource quality and  
512 stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology* 54:957–970.

513 Hooper, D.U., Chapin, F.S., III, Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge,  
514 D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., & Wardle,  
515 D.A. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge.  
516 *Ecological monographs* 75: 3–35.

517 Jennings, D. E., J. J. Duan, D. Bean, J. R. Gould, K. A. Rice, and P. M. Shrewsbury. 2016. Monitoring the  
518 establishment and abundance of introduced parasitoids of emerald ash borer larvae in Maryland,  
519 U.S.A. *Biological control: theory and applications in pest management* 101:138–144.

520 Johnson, A. L., E. C. Tauzer, and C. M. Swan. 2015. Human legacies differentially organize functional  
521 and phylogenetic diversity of urban herbaceous plant communities at multiple spatial scales.  
522 *Applied Vegetation Science* 18:513–527.

523 Jones, J. A., and C. M. Swan. 2016. Community composition and diversity of riparian forests regulate  
524 decomposition of leaf litter in stream ecosystems. *Restoration Ecology* 24:230–234.

525 Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7:  
526 improvements in performance and usability. *Molecular Biology and Evolution* 30:772–780.

527 Laliberte, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from  
528 multiple traits. *Ecology* 91:299–305.

529 Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M.  
 530 Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W  
 531 and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.

532 Lecerf, A., and E. Chauvet. 2008. Intraspecific variability in leaf traits strongly affects alder leaf  
 533 decomposition in a stream. *Basic and Applied Ecology* 9:598–605.

534 Lecerf, A., G. Marie, J. S. Kominoski, C. J. Leroy, C. Bernadet, and C. M. Swan. 2011. Incubation time,  
 535 functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition.  
 536 *Ecology* 92:160–169.

537 Lecerf, A., G. Risnoveanu, C. Popescu, M. O. Gessner, and E. Chauvet. 2007. Decomposition of diverse  
 538 litter mixtures in streams. *Ecology* 88:219–227.

539 LeRoy, C. J., A. L. Hipp, K. Lueders, J. J. Follstad Shah, J. S. Kominoski, M. Ardón, W. K. Dodds, M. O.  
 540 Gessner, N. A. Griffiths, A. Lecerf, D. W. P. Manning, R. L. Sinsabaugh, and J. R. Webster. 2020.  
 541 Plant phylogenetic history explains in-stream decomposition at a global scale. *The Journal of*  
 542 *Ecology* 108:17–35.

543 López-Rojo, N., Pérez, J., Basaguren, A., Pozo, J., Rubio-Ríos, J., Casas, J.J., & Boyero, L. 2020. Effects  
 544 of two measures of riparian plant biodiversity on litter decomposition and associated processes in  
 545 stream microcosms. *Scientific reports* 10: 19682.

546 Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic  
 547 structure of communities. *Ecology Letters* 13:1085–1093.

548 Ostrofsky, M. L. 1993. Effect of tannins on leaf processing and conditioning rates in aquatic ecosystems:  
 549 an empirical approach. *Canadian Journal of Fisheries and Aquatic Sciences* 50:1176–1180.

550 Ostrofsky, M. L. 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic  
 551 processing rates. *Journal of the North American Benthological Society* 16:750–759.

552 Pearse, W. D., and A. Purvis. 2013. phyloGenerator: An automated phylogeny generation tool for  
 553 ecologists. *Methods in Ecology and Evolution* 4:692–698.

554 Petchey, O. L., and K. J. Gaston. 2002. Extinction and the loss of functional diversity. *Proceedings of the*  
 555 *Royal Society B: Biological Sciences* 269:1721–1727.

556 R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical  
 557 Computing, Vienna, Austria.

558 Sanpera-Calbet, I., A. Lecerf, and E. Chauvet. 2009. Leaf diversity influences in-stream litter  
 559 decomposition through effects on shredders. *Freshwater Biology* 54:1671–1682.

560 Sponseller, R. A., and E. F. Benfield. 2001. Influences of land use on leaf breakdown in southern  
 561 Appalachian headwater streams: A multiple-scale analysis. *Journal of the North American*  
 562 *Benthological Society* 20:44–59.

563 Srivastava, D. S., M. W. Cadotte, A. A. M. Macdonald, R. G. Marushia, and N. Mirotnick. 2012.  
 564 Phylogenetic diversity and the functioning of ecosystems. *Ecology Letters* 15:637–648.

565 Srivastava, D. S., B. J. Cardinale, A. L. Downing, J. E. Duffy, C. Jouseau, M. Sankaran, and J. P. Wright.  
 566 2009. Diversity has stronger top-down than bottom-up effects on decomposition. *Ecology*  
 567 90:1073–1083.

568 Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large  
 569 phylogenies. *Bioinformatics* 30:1312–1313.

570 Suberkropp, K., G. L. Godshalk, and M. J. Klug. 1976. Changes in the Chemical Composition of Leaves  
 571 During Processing in a Woodland Stream. *Ecology* 57:720–727.

572 Swan, C. M. 2011. Consumer presence and resource diversity independently induce stability of  
 573 ecosystem function in a Piedmont stream. *Ecosphere* 2:art136.

574 Swan, C. M., M. A. Gluth, and C. L. Horne. 2009a. Leaf litter species evenness influences nonadditive  
 575 breakdown in a headwater stream. *Ecology* 90:1650–1658.

576 Swan, C. M., P. D. Jensen, G. P. Dively, and W. O. Lamp. 2009b. Processing of transgenic crop residues  
 577 in stream ecosystems. *The Journal of Applied Ecology* 46:1304–1313.

578 Swan, C. M., and M. A. Palmer. 2004. Leaf diversity alters litter breakdown in a Piedmont stream. *Journal*  
 579 *of the North American Benthological Society* 23:15–28.

580 Swan, C. M., and M. A. Palmer. 2006a. Preferential feeding by an aquatic consumer mediates non-  
 581 additive decomposition of speciose leaf litter. *Oecologia* 149:107–114.

582 Swan, C. M., and M. A. Palmer. 2006b. Composition of speciose leaf litter alters stream detritivore  
 583 growth, feeding activity and leaf breakdown. *Oecologia* 147:469–478.

584 Sweeney, B. W. 1993. Effects of Streamside Vegetation on Macroinvertebrate Communities of White Clay  
585 Creek in Eastern North America. *Proceedings of the Academy of Natural Sciences of*  
586 *Philadelphia* 144:291–340.

587 Sweeney, B. W., and S. J. Czapka. 2004. Riparian forest restoration: Why each site needs an ecological  
588 prescription. *Forest Ecology and Management* 192:361–373.

589 Talevich, E., B. M. Invergo, P. J. A. Cock, and B. A. Chapman. 2012. Bio.Phylo: a unified toolkit for  
590 processing, analyzing and visualizing phylogenetic trees in Biopython. *BMC Bioinformatics*  
591 13:209.

592 Tiegs, S. D., D. M. Costello, M. W. Isken, G. Woodward, P. B. McIntyre, M. O. Gessner, E. Chauvet, N. A.  
593 Griffiths, A. S. Flecker, V. Acuña, R. Albariño, D. C. Allen, C. Alonso, P. Andino, C. Arango, J.  
594 Aroviita, M. V. M. Barbosa, L. A. Barmuta, C. V. Baxter, T. D. C. Bell, B. Bellinger, L. Boyero, L.  
595 E. Brown, A. Bruder, D. A. Bruesewitz, F. J. Burdon, M. Callisto, C. Canhoto, K. A. Capps, M. M.  
596 Castillo, J. Clapcott, F. Colas, C. Colón-Gaud, J. Cornut, V. Crespo-Pérez, W. F. Cross, J. M.  
597 Culp, M. Danger, O. Dangles, E. de Eyto, A. M. Derry, V. D. Villanueva, M. M. Douglas, A.  
598 Elosegí, A. C. Encalada, S. Entrekin, R. Espinosa, D. Ethaiya, V. Ferreira, C. Ferriol, K. M.  
599 Flanagan, T. Fleituch, J. J. Follstad Shah, A. Frainer Barbosa, N. Friberg, P. C. Frost, E. A.  
600 Garcia, L. García Lago, P. E. García Soto, S. Ghate, D. P. Giling, A. Gilmer, J. F. Gonçalves Jr,  
601 R. K. Gonzales, M. A. S. Graça, M. Grace, H.-P. Grossart, F. Guérol, V. Gulis, L. U. Hepp, S.  
602 Higgins, T. Hishi, J. Huddart, J. Hudson, S. Imberger, C. Iñiguez-Armijos, T. Iwata, D. J. Janetski,  
603 E. Jennings, A. E. Kirkwood, A. A. Koning, S. Kosten, K. A. Kuehn, H. Laudon, P. R. Leavitt, A. L.  
604 Lemes da Silva, S. J. Leroux, C. J. LeRoy, P. J. Lisi, R. MacKenzie, A. M. Marcarelli, F. O.  
605 Masese, B. G. McKie, A. Oliveira Medeiros, K. Meissner, M. Miliša, S. Mishra, Y. Miyake, A.  
606 Moerke, S. Mombrikotb, R. Mooney, T. Moulton, T. Muotka, J. N. Negishi, V. Neres-Lima, M. L.  
607 Nieminen, J. Nimptsch, J. Ondruch, R. Paavola, I. Pardo, C. J. Patrick, E. T. H. M. Peeters, J.  
608 Pozo, C. Pringle, A. Prussian, E. Quenta, A. Quesada, B. Reid, J. S. Richardson, A. Rigosi, J.  
609 Rincón, G. Rîşnoveanu, C. T. Robinson, L. Rodríguez-Gallego, T. V. Royer, J. A. Rusak, A. C.  
610 Santamans, G. B. Selmechy, G. Simiyu, A. Skuja, J. Smykla, K. R. Sridhar, R. Sponseller, A.  
611 Stoler, C. M. Swan, D. Szlag, F. Teixeira-de Mello, J. D. Tonkin, S. Uusheimo, A. M. Veach, S.

- Vilbaste, L. B. M. Vought, C.-P. Wang, J. R. Webster, P. B. Wilson, S. Woelfl, M. A. Xenopoulos, A. G. Yates, C. Yoshimura, C. M. Yule, Y. X. Zhang, and J. A. Zwart. 2019. Global patterns and drivers of ecosystem functioning in rivers and riparian zones. *Science Advances* 5:eaav0486.
- Vellend, M., K. Verheyen, K. M. Flinn, H. Jacquemyn, A. Kolb, H. Van Calster, G. Peterken, B. J. Graae, J. Bellemare, O. Honnay, J. Brunet, M. Wulf, F. Gerhardt, and M. Hermy. 2007. Homogenization of forest plant communities and weakening of species-environment relationships via agricultural land use. *The Journal of Ecology* 95:565–573.
- Villéger, S., S. Blanchet, O. Beauchard, T. Oberdorff, and S. Brosse. 2015. From current distinctiveness to future homogenization of the world's freshwater fish faunas. *Diversity and Distributions* 21:223–235.
- Villéger, S., N. W. H. Mason, and D. Mouillot. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* 89:2290–2301.
- Webb, C. O., D. D. Ackerly, M. A. McPeck, and M. J. Donoghue. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33:475–505.
- Webster, J. R., and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* 17:567–594.



Table 3. Linear regression results relating foliar chemistry and structural attributes of leaf litter employed in the 2014 study on breakdown rate. Breakdown rate is regressed on breakdown.

	<b>Slope</b>	<b>Adjusted <math>r^2</math></b>	<b><math>P_{slope}</math></b>
<b>N</b>	0.009	0.125	0.029
<b>P</b>	0.061	0.154	<0.001
<b>C:N</b>	-7.518e <sup>-05</sup>	0.120	0.084
<b>Toughness</b>	-3.173e <sup>-04</sup>	0.269	<0.001
<b>Phenolics</b>	-0.001	0.158	<0.001

Table 4. General linear model results for both studies. Estimates for each parameters are given with associated standard errors (SE). "Int" indicates an intercept effect.

	2014			2015		
Coefficient	Estimate	SE	t	Estimate	SE	t
Int: Stream A	0.061	0.0088	6.96***	0.065	0.0085	7.67***
Int: Stream B	0.073	0.0088	8.37***	0.061	0.0085	7.19***
Int: Stream C	0.055	0.0088	6.27***	0.039	0.0085	4.62***
Phylogenetic Div	-0.006	0.0040	-0.40	0.003	0.0085	2.67*
Functional Div	-0.002	0.0040	-0.55	0.010	0.0045	2.17*
	<b>F<sub>5,40</sub></b>	111.8***		<b>F<sub>5,43</sub></b>	206.4***	
	<b>Adj r<sup>2</sup></b>	0.92		<b>Adj r<sup>2</sup></b>	0.96	

\* $P < 0.05$ , \*\*\* $P < 0.001$

Table 1. Foliar chemistries and structural characteristics of leaf litter for species employed in both studies. Abbreviations in parentheses are to aid in identifying species in mixtures.

Species	2014					2015				
	C:N	Toughness*	Phenolics**	% P	% N	C:N	Toughness*	Phenolics**	% P	% N
<i>Acer negundo</i> (AN)	33.3	139.2	23.89	0.093	1.297					
<i>Acer platanoides</i> (AP)	60.8	116.1	40.86	0.097	0.752					
<i>Acer rubrum</i> (AR)	136.1	120.6	38.61	0.167	0.335	243.3	152.7	38.6	0.167	0.233
<i>Acer saccharum</i> (AS)	93.7	138.2	18.81	0.214	0.485	93.9	174.9	18.8	0.214	0.526
<i>Betula lenta</i> (BL)	60.3	228.8	42.41	0.383	0.596	108.3	289.6	42.4	0.383	0.494
<i>Betula nigra</i> (BN)	43.3	114.9	24.15	0.052	0.802	62.9	145.4	24.2	0.052	0.869
<i>Carya glabra</i> (CG)	61.3	166.5	39.96	0.091	0.613	90.6	210.8	40.0	0.091	0.554
<i>Cornus florida</i> (CF)	31.2	114.5	12.60	0.075	0.957	105.9	144.9	12.6	0.075	0.492
<i>Fagus grandifolia</i> (FG)	48.5	130.8	29.26	0.033	0.656	150.7	165.6	29.3	0.033	0.342
<i>Fraxinus pennsylvanica</i> (FP)	29.7	109.2	12.08	0.115	1.099	81.9	138.2	12.1	0.115	0.663
<i>Juglans nigra</i> (JN)	31.5	99.4	17.91	0.082	1.107	132.2	125.8	17.9	0.082	0.415
<i>Liquidambar styraciflua</i> (LS)	145.4	187.1	24.69	0.222	0.334	171.6	236.9	24.7	0.222	0.282
<i>Liriodendron tulipifera</i> (LT)	98.1	194.5	34.78	0.087	0.470	76.5	246.2	34.8	0.087	0.591
<i>Nyssa sylvatica</i> (NS)	79.1	151.6	33.65	0.230	0.588	110.1	191.9	33.7	0.230	0.415
<i>Platanus occidentalis</i> (PO)	40.4	191.6	22.98	0.084	0.804	85.5	242.6	23.0	0.084	0.624
<i>Quercus alba</i> (QA)	77.9	236.1	45.27	0.087	0.539	100.8	298.9	45.3	0.087	0.481
<i>Quercus bicolor</i> (QB)	57.3	248.2	36.23	0.155	0.657		0.0			
<i>Quercus coccinea</i> (QC)	100.6	273.1	33.48	0.048	0.476		0.0			
<i>Quercus falcata</i> (QF)	60.4	206.3	40.32	0.049	0.730	97.3	261.2	40.3	0.049	0.590
<i>Quercus nigra</i> (QN)	63.5	331.0	27.27	0.020	0.784	70.8	419.0	27.3	0.020	0.705
<i>Quercus palustris</i> (QPa)	48.8	167.3	27.61	0.047	0.924		0.0			
<i>Quercus phellos</i> (QPh)	56.5	167.3	36.15	0.067	0.701	146.0	211.8	36.2	0.067	0.383
<i>Quercus prinus</i> (QPr)	63.8	272.7	40.45	0.042	0.763	100.0	345.1	40.5	0.042	0.520
<i>Quercus rubra</i> (QR)	50.8	306.5	22.35	0.088	0.980		0.0			

<i>Quercus velutina</i> (QV)	50.9	247.3	30.53	0.089	0.865		0.0			
<i>Robinia pseudoacacia</i> (RP)	23.6	109.6	18.34	0.037	1.899		0.0			
<i>Salix nigra</i> (SN)	38.3	160.0	42.02	0.063	1.309		0.0			
<i>Sassafras albidum</i> (SA)	55.8	189.4	32.09	0.049	0.866	82.5	239.7	32.1	0.049	0.665
<i>Ulmus americana</i> (UA)	36.4	142.2	27.95	0.404	0.891					
<i>Ulmus rubra</i> (UR)	31.7	89.2	18.98	0.084	1.161					

---

\* g mm<sup>-2</sup>, \*\* μg g<sup>-1</sup>

---

677

678

679

680

681

682

683

684

685

686

687

688

689

690

691

Table 2. Phylogenetic and Functional Diversity of each mixture for 2014 and 2015. Shannon Diversity is given for the 2014 study for reference; it is not given for 2014 as it was held constant across all treatments.

<b>2014</b>	<b>Phylogenetic Diversity (MPD)</b>	<b>Functional Diversity (FDis)</b>	<b>Shannon Diversity (H')</b>
BL-AS	0.13	0.20	0.13
AR-FP	0.29	1.97	0.61
LS-CF-LT	0.33	0.23	0.22
BN-AR-LS	0.24	1.09	0.81
FG-CG-AR	0.23	1.47	0.93
QC-QR-CG-LT	0.51	0.44	0.72
QA-FG-NS-LT	0.29	0.89	0.73
AR-LS-FP-NS	0.36	2.12	0.96
QR-FG-PO-LT	0.51	0.93	1.09
QA-CG-AR-LS-LT	0.63	0.86	1.11
QR-CG-RP-AR-LT	0.40	1.92	1.29
QA-AR-LS-FP-LT	0.63	1.41	1.44
QPr-BL-AR-NS-SA	0.49	2.13	1.44
QC-QA-FG-AR-LS-LT	0.53	0.92	1.24
QA-FG-AR-LS-NS-LT	0.70	1.25	1.53
<b>2015</b>			
QPh-QF-QA-QPr	-5.14	0.95	
QF-QPr-BN-CG	-2.11	1.36	
QA-QPr-CG-AS	-1.37	1.41	
BN-JN-FP-NS	-2.21	1.73	
BN-BL-CG-LT	-3.59	1.80	
AS-FP-NS-CF	-3.57	1.24	
LS-FP-NS-CF	-3.97	1.70	
BL-CG-JN-AR	-4.25	2.39	
QF-CG-PO-SA	0.80	0.79	
QF-CG-NS-SA	0.79	1.04	
QF-FG-PO-LT	0.83	1.23	
AR-NS-PO-LT	0.87	1.98	
AR-FP-PO-LT	0.88	2.16	
BL-LS-PO-SA	0.82	2.06	
QA-LS-FP-SA	0.81	2.01	
QN-BL-CF-LT	0.85	2.25	
QPh-QF-QA-QPr	-5.14	0.95	

Figure 1. Phylogenetic tree of tree species with tissue chemistries and breakdown mapped. Darker colors indicate relatively higher values; all values were standardized from 0-1 and colors ranked accordingly.

Figure 2. 2014 breakdown of all single species leaf litters. Mean plus pooled errors bars from randomized block ANOVA.

Figure 3. 2014 breakdown versus phylogenetic diversity (a) and functional diversity (b). Neither relationship is significant. (c) Observed breakdown of mixed litter averaged across replicates within stream versus the predicted breakdown based on the weighted average of the single species breakdown rates in those same streams. The dashed line indicated the 1:1 relationship whereby observed breakdown is equal to predicted breakdown, and therefore no effect of mixing. Values below this line suggest mixing leaf species slow breakdown rate (dark circles); above accelerates breakdown (open circles).

Figure 4. As is Figure 3, breakdown rates of litter mixtures versus phylogenetic diversity (a) and functional diversity (b) for the 2015 study. Both relationships are positive and significant. (c) Observed breakdown of mixed litter averaged across replicates within stream versus the predicted breakdown based on the average of the four single species breakdown rates in those same streams. The dashed line indicated the 1:1 relationship whereby observed breakdown is equal to predicted breakdown, and therefore no effect of mixing. Values below this line suggest mixing leaf species slow breakdown rate (dark circles); above accelerates breakdown (open circles).

Figure 1

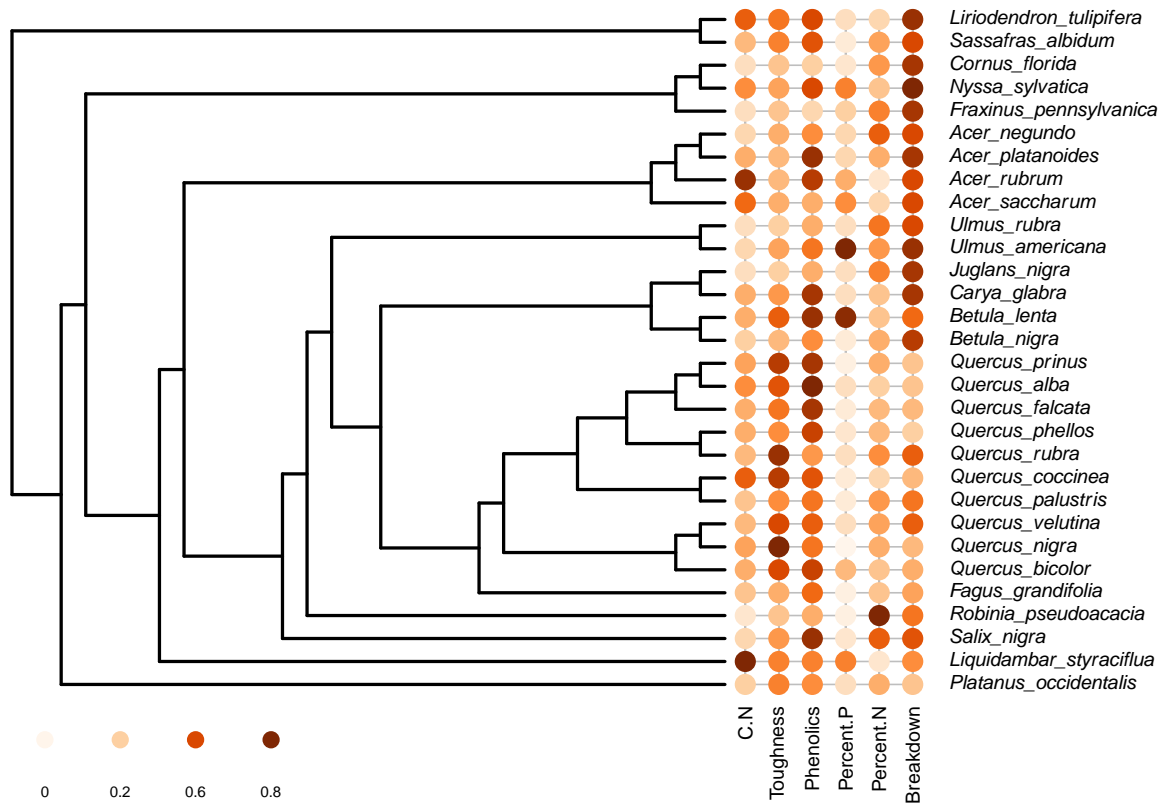


Figure 2

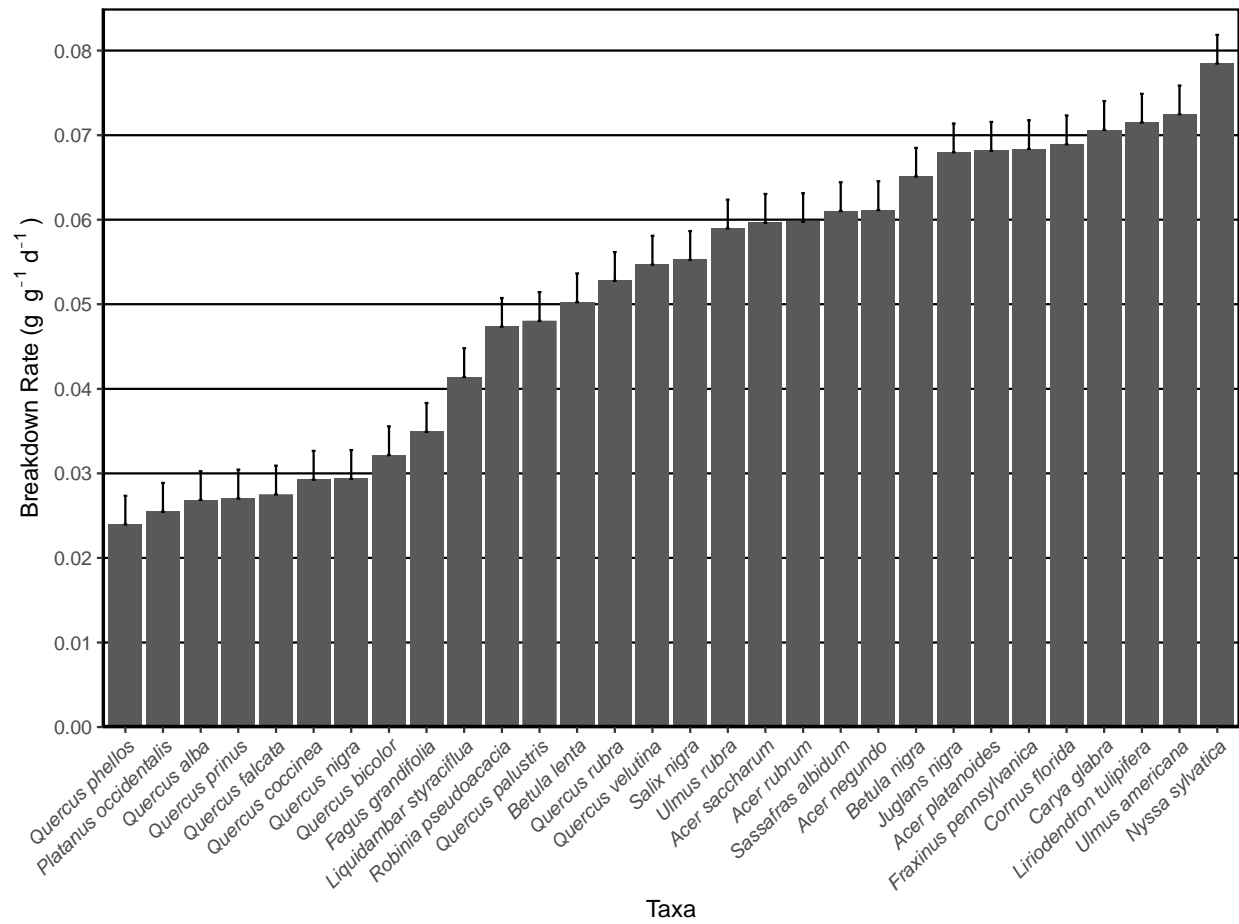
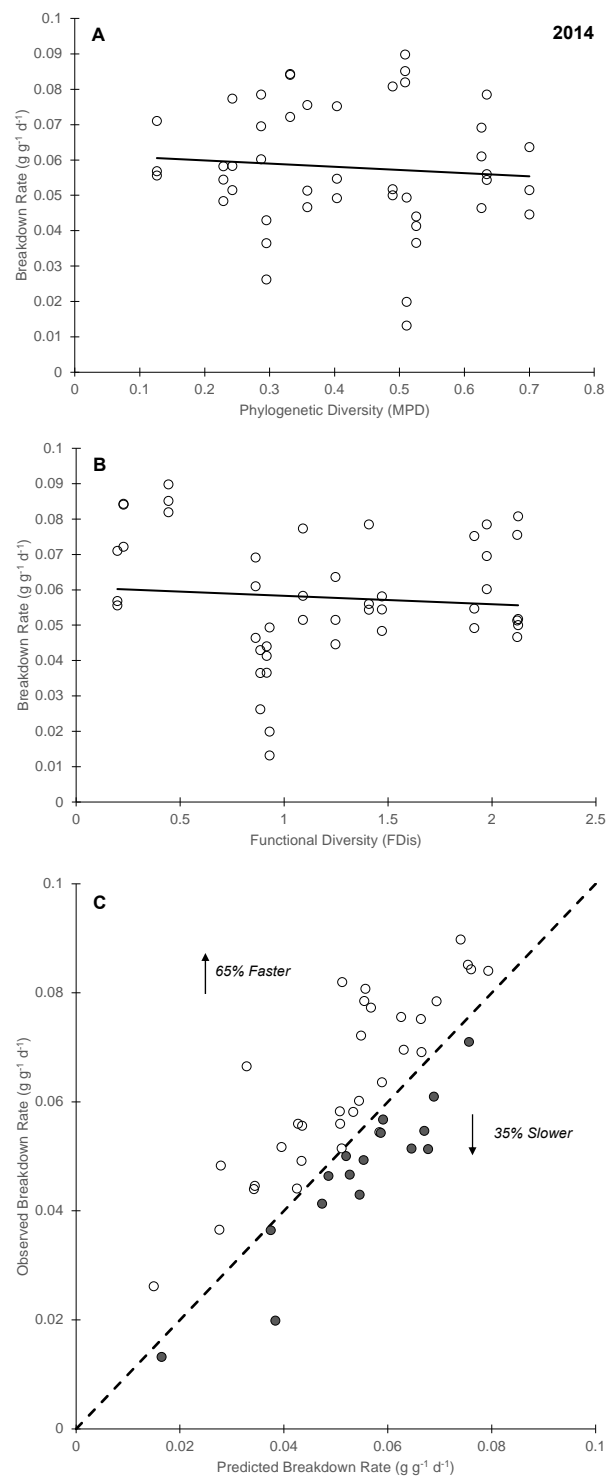


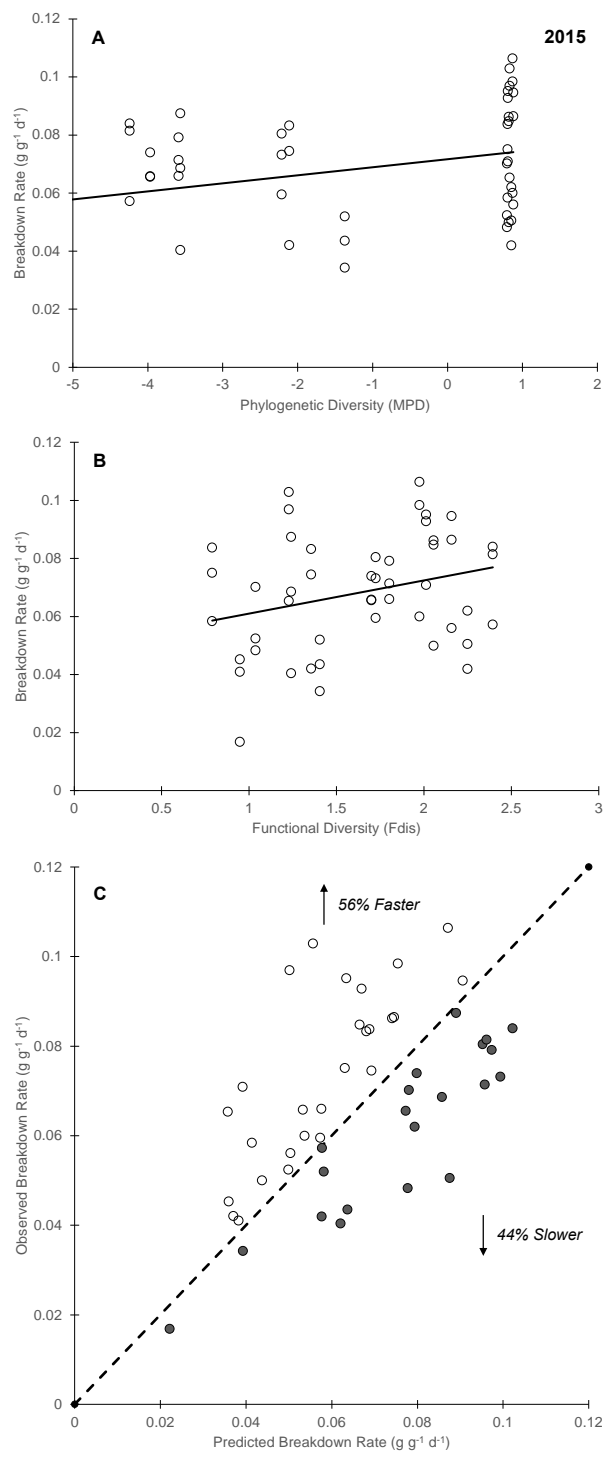


Figure 3



751

752 Figure 4



753

754

755