Global Patterns and Controls of Nutrient Immobilization on Decomposing Cellulose in Riverine Ecosystems

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Key Points:

- Nitrogen and phosphorus immobilization was measured on organic matter (cotton) in 100 rivers and riparian zones representing 11 biomes
- Elevated temperature in riparian zones and phosphate in rivers increased immobilization, and consequently accelerated decomposition
- Nitrogen and phosphorus immobilization was strongly linked by microbial stoichiometry despite widely varied surface-water nutrient ratios

Abstract

Microbes play a critical role in plant litter decomposition and influence the fate of carbon in rivers and riparian zones. When decomposing low-nutrient plant litter, microbes acquire nitrogen (N) and phosphorus (P) from the environment (i.e., nutrient immobilization), and this process is potentially sensitive to nutrient loading and changing climate. Nonetheless, environmental controls on immobilization are poorly understood because rates are also influenced by plant litter chemistry, which is coupled to the same environmental factors. Here we used a standardized, low-nutrient organic matter substrate (cotton strips) to quantify nutrient immobilization at 100 paired stream and riparian sites representing 11 biomes worldwide. Immobilization rates varied by 3 orders of magnitude, were greater in rivers than riparian zones, and were strongly correlated to decomposition rates. In rivers, P immobilization rates were controlled by surface water phosphate concentrations, but N immobilization rates were not related to inorganic nitrogen. The N:P of immobilized nutrients was tightly constrained to a molar ratio of 10:1 despite wide variation in surface water N:P. Immobilization rates were temperature-dependent in riparian zones but not related to temperature in rivers. However, in rivers nutrient supply ultimately controlled whether microbes could achieve the maximum expected decomposition at a given temperature. Collectively, we demonstrated that exogenous nutrient supply and immobilization are critical control points for decomposition of organic matter.

Plain Language Summary

Bacteria and fungi contribute to the breakdown of leaf litter in rivers and floodplains. To break down leaf litter, these microbes need the nutrients nitrogen and phosphorus, and microbes can get nutrients either from the leaf litter itself or from the environment. Most leaf litter has low nutrient content and microbes must rely on the environment to supply nutrients. We studied

microbial nutrient uptake from the environment during litter breakdown to determine whether it varies predictably across the globe and how it is influenced by changing climate and nutrient pollution. In 100 rivers and floodplains in 11 of Earth's major biomes we placed small strips of cotton as stand-ins for leaf litter. Nutrient uptake was consistently greater on cotton strips that were submerged in the river compared to cotton on the floodplain. For microbes in the river, nutrient uptake was faster in instances where there was more phosphorus in the water. For microbes in the floodplain, nutrient uptake was faster where temperatures were warmer. Faster nutrient uptake by microbes was linked with faster cotton breakdown in rivers and floodplains. Our study shows that climate change and nutrient pollution can alter the activity of microbes in rivers and floodplains.

1 Introduction

The uptake, storage, transformation, and release of nutrients by microorganisms are among the most important contributions that riverine ecosystems make to global biogeochemical cycles. However, microbially mediated nutrient uptake and transformation are influenced by environmental conditions, which makes these processes particularly sensitive to global change drivers including rising temperature, nutrient loading, and chemical contaminants (Boyero et al., 2011; Burdon et al., 2020; Woodward et al., 2012). Detrital carbon (C) from terrestrial plants is abundant in riparian zones and rivers (Sutfin et al., 2016; Tank et al., 2010) and this resource fuels microbial communities and associated nutrient uptake and transformation across the terrestrial–aquatic boundary. Detrital C is used by microbial decomposers most efficiently when nitrogen (N) and phosphorus (P) are readily available; however, pools of N and P can differ between riparian and river habitats and are increasing worldwide, with potential implications for

rates of organic-matter mineralization. In both habitats, the dynamics of N and P on decomposing plant litter have shown characteristic patterns (Manzoni et al., 2010; Webster & Benfield, 1986). Early in decomposition of carbon-rich materials, nutrients are acquired by microbes from the environment, resulting in a net increase in microbial pools of N and P above that supplied from the litter itself (i.e., nutrient immobilization), whereas in later stages, there is a net release of nutrient mass from microbial pools (i.e., nutrient mineralization) as substrates degrade. Nutrient mineralization has received more attention than nutrient immobilization, and litter chemistry (e.g., C:N, lignin content) appears to be the dominant driver of mineralization rates (Manzoni et al., 2010; Parton et al., 2007).

Current understanding of the controls on nutrient uptake and release during plant litter decomposition of plant matter begins with litter chemistry, which varies widely among plant species (Boyero et al., 2017; Parton et al., 2007). Exogenous factors such as climate and soil nutrient concentrations are predicted to indirectly influence microbial nutrient dynamics through changes in litter quality (Classen et al., 2015; Manzoni et al., 2008). However, comparative studies of nutrient dynamics during decomposition at broad spatial scales have mostly used natural litter, potentially confounding the chemistry of plant litter with the environmental factors that produced it (Boyero et al., 2017; Pastor et al., 2014). Standardized organic-matter substrates allow for stronger inferences about the role of exogenous factors in governing nutrient immobilization by the microbes that drive decomposition. However, the use of standardized organic matter substrates is commonly used at small spatial scales along narrow nutrient gradients (e.g., Cheever et al., 2013; Pastor et al., 2014) and the only large-scale study using standardized substrates did not directly measure microbial uptake (Woodward et al., 2012). Few studies have quantified both N and P immobilization within the same decomposing substrate (but

see Robbins et al., 2019) thus we have limited evidence about the stoichiometry of immobilization. Bacteria (inclusive of all decomposer prokaryotes) and fungi are the primary decomposers of plant litter, and both taxa can produce biomass with an N:P reflective of the nutrient supply (Danger & Chauvet, 2013; Godwin & Cotner, 2015). Thus, we may expect substantial flexibility in the N:P of new decomposer biomass, resulting in N:P of immobilization that is related to the exogenous nutrient supply. Furthermore, microbial uptake of nutrients, like other metabolic processes, may be constrained by temperature (e.g., Brown et al., 2004, Follstad Shah et al. 2017) and affected by moisture availability (Boyero et al., 2011). Therefore, studies of how homogenous substrates decompose along broad climatic gradients and outside the wetted channel are needed to gain understanding about a more complete set of exogenous drivers.

To isolate how exogenous drivers influence nutrient dynamics during litter decomposition, we conducted a field experiment in which we deployed a standard organic substrate (cotton) in 100 rivers and their riparian zones distributed across Earth's major biomes and measured nutrient immobilization potential. Cotton is composed almost entirely of cellulose (Tiegs et al., 2013), and natural sources of plant litter also have substantial cellulose content (e.g., 40% for common oak species in Europe; Fioretto et al., 2005). This nutrient-poor organic-matter substrate obligates microbes to immobilize N and P in order to respire the cellulose. The rate of immobilization, and thus indirectly the rate of cotton decomposition, is limited by the availability of nutrients in the environment. Nutrient availability differs along the river–riparian boundary, and we hypothesized that immobilization in riparian zones would be less than in adjacent rivers due to intermittent fluxes of water and nutrients. We hypothesized that immobilization rates would be governed by ambient temperature in accordance with metabolic scaling (Brown et al., 2004). Thus, we predicted that global patterns of nutrient immobilization rates and ratios could

be predicted from latitude and terrestrial biome classification as proxies for climate (Dodds et al. 2019). Finally, we hypothesized that coupling between N and P immobilization would be weak as the bacteria and fungi that colonize and decompose cotton (Burdon et al., 2020; Colas et al., 2019) are stoichiometrically flexible.

2 Materials and Methods

2.1 Field sites

A standardized decomposition assay using cotton strips was implemented in more than 500 riverine ecosystems by the Cellulose Decomposition Experiment (CELLDEX) (Tiegs et al., 2019). Partners completed the decomposition assay in rivers and their adjacent riparian zone during peak litterfall. When possible, they logged ambient temperature (hourly) in both habitats (details in Tiegs et al. 2019) and when deploying cotton strips recorded specific conductance, ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), and dissolved organic carbon (DOC) concentrations. Surface water PO_4^{3-} and NO_3^- spanned three orders of magnitude, which was similar to the gradient in nutrient concentrations observed in the only other large scale study of nutrient effects on decomposition (Woodward et al., 2012). Measurements of nutrient immobilization were made for a subset of the CELLDEX sites (99 rivers and 100 riparian zones), with priority given to sites with supporting data on river temperature and nutrient concentrations (50–71% of sites, depending on analyte, Text S1). If >10 sites within a biome had supporting data, 10 of them were randomly selected. The resulting subset represented 11 biomes with at least two rivers in each biome (with the exception of deserts) that had NO_3^- and PO_4^{3-}

measurements (Figure S1). Paired rivers and riparian zones were included in the subsample with the exception of a river in a tropical wet forest where high flows caused loss of the strips.

2.2 Cotton deployment and analyses

Cotton strips were used as a low-nutrient analogue for leaf litter. The standardized material was composed of 95% cellulose with 7180 µg N g^{-1} dry mass (dm) and 64 µg P g^{-1} dm. The molar stoichiometry of cotton (C:N = 275, C:P = 17,000) suggests large nutrient deficiencies (relative to C) near the maxima observed in natural leaf litter (Manzoni et al., 2010; McGroddy et al., 2004). However, the N:P ratio of 62:1 was similar to that observed for some tropical leaf litter (Boyero et al., 2017; McGroddy et al., 2004). We expected that the cotton strips would be colonized by heterotrophic bacteria and fungi, and growth of autotrophs was limited by shading from riparian vegetation in most of our rivers (channel width <5 m at 80% of sites). Strips were prepared according to published methods (Tiegs et al., 2013, 2019) and 4 replicate strips per river channel and 4 per riparian zone were deployed. In the river, strips were attached to nylon rope with cable ties and secured to a stake. In the riparian zone, strips were placed in contact with the soil or surface organic matter. Deployment lasted approximately 3-4 weeks (range 12-57 d), which is a sufficient period to detect microbial degradation (as loss of tensile strength) in both habitats (Tiegs et al., 2019). Upon retrieval, strips were placed in ethanol (<1 min) to arrest microbial activity and dried (40 °C) before analysis. The brief submergence in ethanol had no effect on nutrient concentrations (Figure S2).

Decomposition during incubation in the field was assessed as loss of tensile strength, resulting from the microbial degradation of cellulose (Tiegs et al., 2013), as reported by Tiegs and colleagues (2019). Strips were subsequently stored in a desiccator until reaching a stable mass and then subsampled with a paper punch which removed small disks from the centerline of the

strips. Cotton disks were acid digested to measure P concentration, and C and N concentration was measured with an elemental analyzer. Detailed methods, recovery of standard reference materials, and procedural reproducibility are described in the Supporting Information (Text S1, Table S1).

2.3 Data analysis

N and P immobilization was calculated based on the assumption that C:N and C:P of decomposing litter decreases linearly as a function of mass loss (Aber & Melillo, 1982; Manzoni et al., 2010). We assumed that any increase in nutrient concentration was a result of assimilation by heterotrophic microbes, but we acknowledge that other processes may also contribute to changes in cotton nutrient content (e.g., algal colonization, P precipitation). Algal assimilation is a relatively minor contribution to net nutrient uptake on detritus except under high light and high nutrient conditions (Elosegi et al. 2018; Halvorson et al. 2019). If litter has a high initial C:nutrient ratio, linear changes in litter stoichiometry result in curvilinear trajectories in total N and P mass as a function of mass remaining, where N and P are initially net immobilized (i.e., nutrient mass increases) until reaching a peak when net nutrient mineralization starts (Aber & Melillo, 1982; Berendse et al., 1987; Manzoni et al., 2008). We used the initial and final C content of disks punched from the cotton strips to estimate mass loss and the C:N and C:P ratios to calculate the maximum nutrient mass immobilized (N and P factors), the rate at which N and P were immobilized (N_{IMM} and P_{IMM}, respectively), and the length of time that cotton immobilized nutrients (T_{IMM}). See Supporting Information for details of the calculations (Text S1). Nutrient immobilization rates, factors, and T_{IMM} were log-normally distributed with a positive skew, and thus all rates, factors, ratios, and times were summarized as geometric means (Isles, 2020). It was not possible to calculate N_{IMM} and P_{IMM} when the substrate had no detectable carbon loss, and

thus some strips could not generate estimates of immobilization (7 and 15% of the river and riparian strips). Additionally, estimates of T_{IMM} and nutrient factors (but not N_{IMM} and P_{IMM}) for strips showing minimal, although detectable, carbon mass loss were insufficiently precise and hence were also excluded from all analyses (Text S1, Figures S3–4).

Nutrient immobilization factors and rates were calculated for individual cotton strips and compared among biomes and latitude using linear mixed models with site as a random effect. For strips that immobilized both N and P (68 rivers and 57 riparian zones), we calculated N:P of immobilization on individual strips and tested for differences among biomes and latitude using linear mixed models. A single decomposition rate (rate of tensile-strength loss, *k*) was estimated at each river and riparian site; thus, *k* was correlated with mean immobilization rate from all strips in a river or riparian zone. Mean immobilization rates in rivers were related to measures of water quality using linear regression. Arrhenius plots were used to examine temperature sensitivity of nutrient immobilization rates in riparian and river sites (separate mixed effects models) with directly measured mean daily temperature during the incubation period. Immobilization rates were also regressed against the deviations (i.e., residuals) from the mean rate of tensile-strength loss (Brown et al., 2004), which were calculated from Arrhenius plot best-fit lines using the complete dataset of Tiegs and colleagues (2019). All statistical analyses were completed in R version 4.0.2 (R Core Team, 2020) and mixed models were fit using the lme4 package.

3 Results

In all 99 rivers, the decomposer community immobilized P while breaking down cotton. In contrast, N immobilization was detectable in only 68 rivers. Of the 98 sites where strip mass was lost in riparian zones, each site immobilized P during breakdown, but N was immobilized at only

35 sites. Globally, nutrient immobilization was faster in rivers than in riparian zones with the geometric mean (and interquartile ranges (IQR)) of 9.3 (4.4–19.7) and 4.8 (3.1–9.4) μg P g^{-1} C d^{-1}, respectively. N immobilization showed an even larger discrepancy between river (72 μg N g^{-1} C d^{-1}; IQR 32–277) and riparian habitats (16 μg N g^{-1} C d^{-1}; IQR 17–74). On average, decomposers were projected to immobilize P in rivers and riparian zones for 78 and 82 days (i.e., T_{IMM}), respectively, before net mineralization started. In contrast, N was predicted to be immobilized in rivers and riparian zones for just 58 and 46 days, respectively.

3.1 Biome as a predictor of nutrient immobilization

Nutrient immobilization among rivers was highly variable, but this variation was only minimally explained by the biome classification. Mean N_{IMM} was greatest in rivers of temperate broadleaf forests (85 μg N g^{-1} C d^{-1}) and least in rivers in mediterranean regions (1.8 μg N g^{-1} C d^{-1}) (Figure 1a), yet biome explained only 13% of the variation in N_{IMM} across all cotton strips (Figure S5). The N factor was similar among biomes, with slightly more variance explained by biome classification (19%, Figure S5). Biome offered slightly more explanatory power for P_{IMM} (19%) than N_{IMM}, with the slowest rates in mediterranean rivers (3.7 μg P g^{-1} C d^{-1}) and significantly faster rates in rivers in desert, temperate grassland, and tropical wet forest biomes (19, 19, 16 μg P g^{-1} C d^{-1}, respectively, Figure 1b). In rivers, P factor exhibited similar biome patterns to P_{IMM} (18% variance explained).

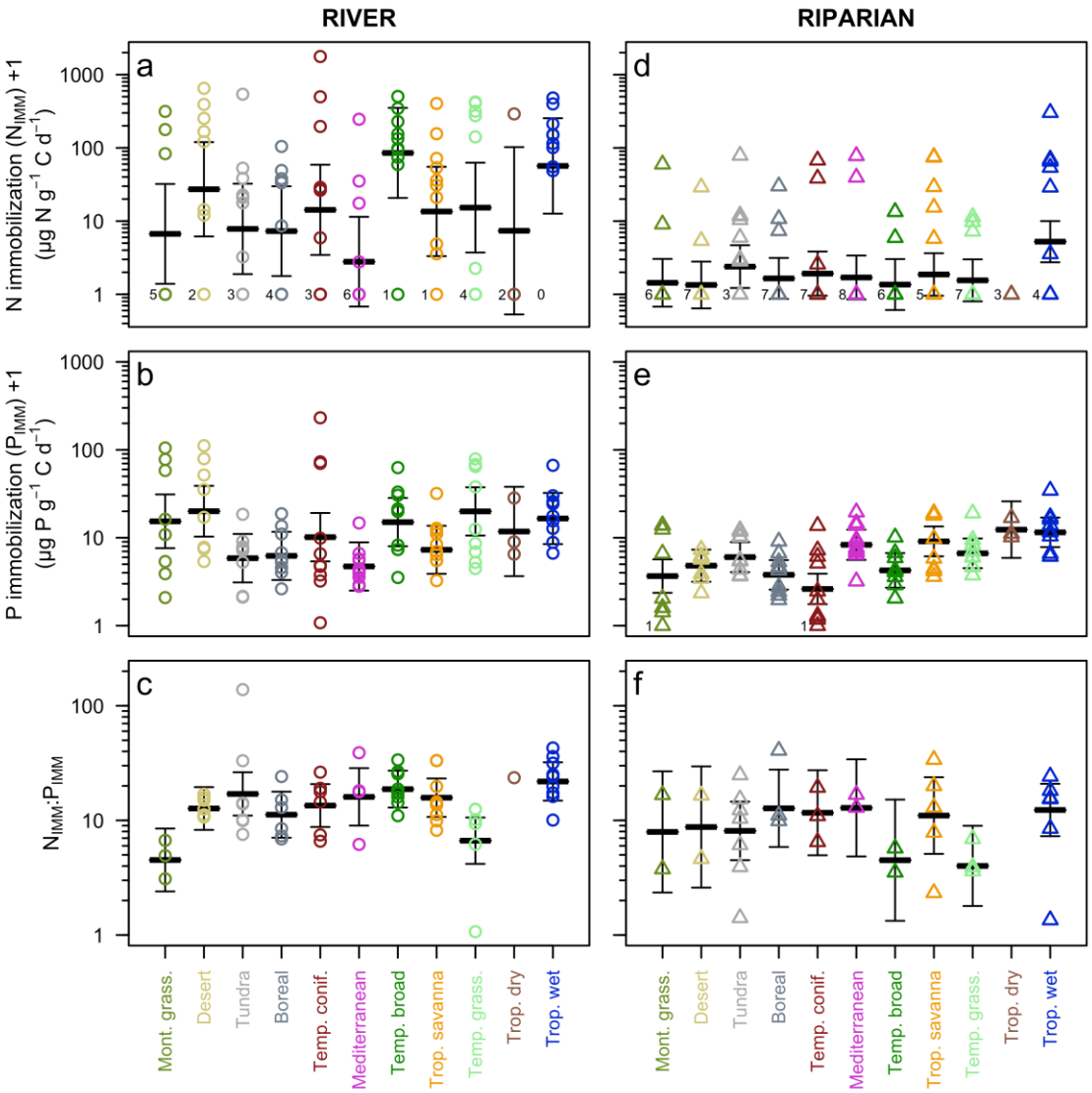

Figure 1. Nitrogen and phosphorus immobilization rates (N_{IMM} and P_{IMM}, respectively) and the molar ratio of the two rates from rivers (a-c) and riparian zones (d-f) in 11 biomes. Black bars indicate means for biomes ±95% confidence intervals. Rates are shown on a log+1 axis to include sites where nutrients were not immobilized during cotton decomposition (count of sites with N_{IMM} or P_{IMM} = 0 are given near x-axis). Biomes are ordered from slowest to fastest mean decomposition rate in riparian strips (Tiegs et al. 2019).

In rivers where both N and P were immobilized (n = 68), the N:P ratio of immobilization (i.e., N_{IMM}:P_{IMM}) was always >1, with 31% of the variation explained by biome type (Figure 1c, Figure S5). Rivers in montane and temperate grasslands had the lowest N_{IMM}:P_{IMM} (5 and 7, respectively), whereas tropical dry, tropical wet, and temperate broadleaf forests had the highest N_{IMM}:P_{IMM} (24, 22, and 19 respectively). The N factor:P factor did not differ substantially among biomes (Figure S5), but the relationship between N factor and P factor in log-log space showed a proportional relationship (slope = 1).

Nitrogen was not immobilized (N_{IMM} = 0) in 63% of the riparian sites with a null value observed in at least one site from each biome (Figure 1d). Biome type did not predict riparian N_{IMM}, likely due to high within-site variation (Figure S5). On average, the rate of N_{IMM} in riparian zones was 0.9 µg N g^{-1} C d^{-1}. The N factor was greatest in tropical wet forests and similar among all other biomes. P immobilization was measured in all but two riparian sites that had detectable mass loss, and 30 and 23% of the variation in P_{IMM} and P factor was explained by biome, respectively. In general, tropical riparian zones had faster rates of P_{IMM} than temperate zones (Figure 1e). Tropical dry and tropical wet forest riparian zones had the fastest rates of P_{IMM} (both 11 µg P g^{-1} C d^{-1}) and temperate coniferous forests had the slowest (1.6 µg P g^{-1} C d^{-1}). P_{IMM} was only greater than N_{IMM} in the 61 sites where N_{IMM} was 0, but for the riparian zones from sites where N_{IMM} and P_{IMM} were both detectable, rates of N_{IMM} always exceeded P_{IMM} (Figure 1f). Biome was not predictive of N_{IMM}:P_{IMM}, which averaged 9:1 globally.

3.2 Immobilization and cellulose decomposition

There was a strong positive association between rates of nutrient immobilization and *k* in both habitats but the relationship was much stronger in rivers than riparian zones (Figure 2). In rivers and riparian zones, sites with slow decomposition more frequently did not immobilize any N

(i.e., N_{IMM} = 0) (Figure 2a & b). In riparian zones, *k* was weakly associated with N_{IMM} (Figure 2b) but there was a stronger correlation in rivers (Figure 2a). Decomposition was correlated positively to P_{IMM}, but similar to N, relationships were stronger in rivers (Figure 2c & d).

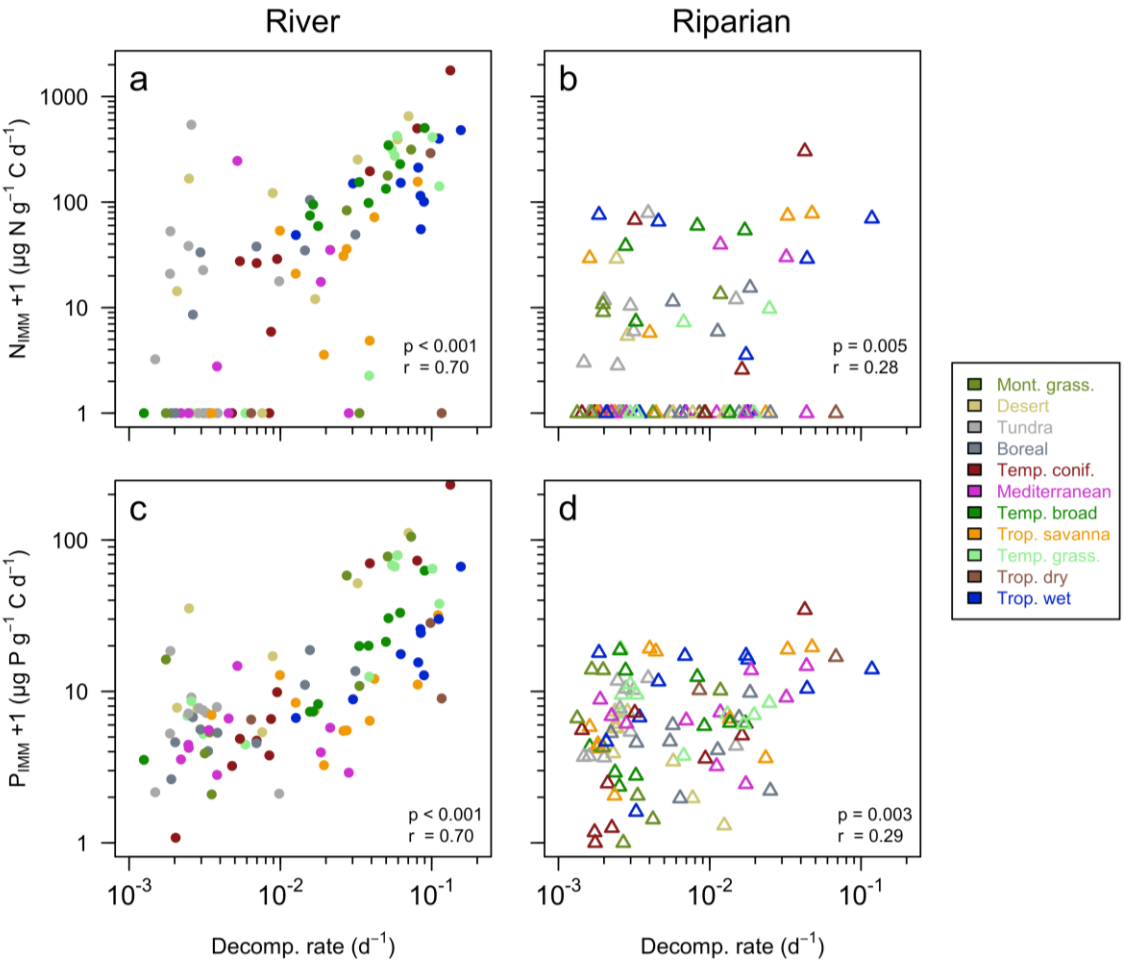

Figure 2. Decomposition rates are positively associated with nitrogen (a & b) and phosphorus (c & d) immobilization rates in rivers (a & c) and riparian zones (b & d). Immobilization rates are on a log+1 axis to include sites where nutrients were not immobilized during decomposition. Immobilization rates plotted are means of 2-4 cotton strips at each site. Decomposition rates (loss of tensile strength) were calculated at the site scale with 2-4 cotton strips (see Tiegs et al. 2019). Symbol colors correspond to different biomes.

3.3 Water quality and immobilization

Some water quality parameters exhibited broad geographic patterns (Text S2) – most notably a negative correlation between PO_4^{3-} and absolute latitude ($p = 0.001$) and greater concentrations of NH_4^+ in temperate grasslands ($p = 0.02$). However, NO_3^-, dissolved inorganic N (DIN), and DIN:PO_4^{3-} did not show any geographic patterns. River water PO_4^{3-} was the only variable significantly related to k ($p = 0.002$). There was also a strong relationship between P_{IMM} and PO_4^{3-} concentrations (Figure 3b). In contrast, neither k ($p = 0.27$) nor N_{IMM} ($p = 0.46$) were correlated with DIN (Figure 3a). For instance, we documented rivers with relatively high DIN (i.e., >100 µg L^{-1}) that did not immobilize N and rivers with relatively low DIN (i.e., <10 µg L^{-1}) where N_{IMM} was in excess of 100 µg N g^{-1} C d^{-1}. The lack of a relationship between surface water N and N_{IMM} was also reflected in poor relationships between NH_4^+ and N_{IMM} ($p = 0.68$), surface water DIN:PO_4^{3-} and N_{IMM}:P_{IMM} (Figure 3c), and NH_4^+:PO_4^{3-} and N_{IMM}:P_{IMM} ($p = 0.75$). Interestingly, there was a weak postive relationship between N_{IMM} and PO_4^{3-} concentrations ($p = 0.06$). Notably, N_{IMM}:P_{IMM} were constrained to a relatively narrow range (50% of N_{IMM}:P_{IMM} between 10 and 20), whereas surface water N:P varied by 3 orders of magnitude (50% of DIN:PO_4^{3-} between 4 and 194).

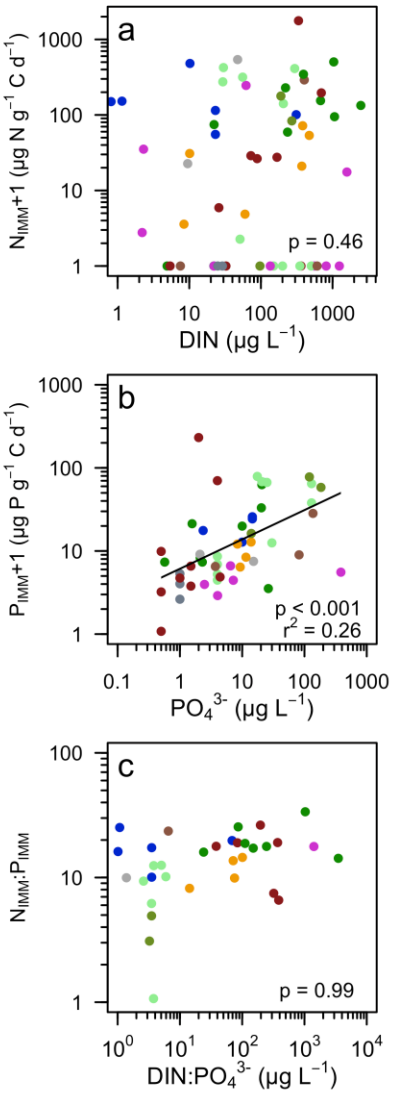

Figure 3. Relationships between ambient surface water nutrients and nutrient immobilization rates by the microbial community on cotton incubated in rivers. DIN is the sum of ammonium-N and nitrate-N concentrations. N:P for surface water and immobilization are molar ratios. Colors denote rivers from different biomes (see legend in Fig. 2).

3.4 Temperature and immobilization

Arrhenius plots of the entire dataset of all sites (n = 415 river and n = 533 riparian sites) indicated strong temperature dependence of *k* in rivers, but only a weak relationship in riparian zones (Tiegs et al., 2019). The sites used in the current analysis reflected the same relationships between *k* and temperature as the larger dataset (Tiegs et al., 2019, Figure S7), which suggests

little bias in our site selection. Nutrient immobilization exhibited strong temperature dependence in riparian sites (slopes: N_{IMM} = -0.52, P_{IMM} = -0.49), but not in rivers (slopes: N_{IMM} = -0.01, P_{IMM} = -0.12) (Figure 4). The contrasting temperature effects on riparian and river habitats were reflected in latitudinal patterns in immobilization (Text S2, Figure S8).

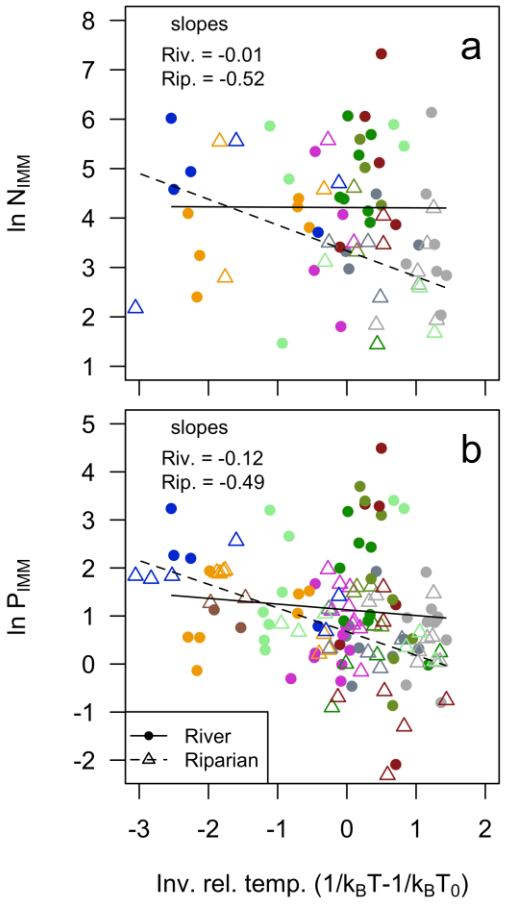

Figure 4. Temperature dependence of nitrogen (a) and phosphorus (b) immobilization on cotton in rivers and riparian zones. Immobilization in riparian zones increased with increasing temperature ($p < 0.02$) but immobilization in rivers was not related to temperature ($p > 0.40$). Colors denote rivers and riparian zones from different biomes (see legend in Fig. 2).

Collectively, our data indicate that *k* was sensitive to temperature, especially in rivers, but there was substantial variation among individual sites with similar temperatures (Figure S7, Tiegs et al., 2019). Deviations in ln(*k*) from the expected value (based on Tiegs et al., 2019) were strongly related to immobilization rates in rivers, but not in riparian zones (Figure 5). In rivers, sites with positive deviations from the temperature relationship (i.e., faster *k* than expected) were characterized by faster rates of N_{IMM} and P_{IMM} , whereas the opposite was true for sites with slower *k* than expected (Figure 5). In contrast, N_{IMM} only weakly explained deviations in ln(*k*) in riparian zones (Figure 5a), particularly when we excluded all sites with $N_{IMM} = 0$ ($p = 0.24$, $r^2 < 0.06$). Similarly, riparian P_{IMM} did not significantly explain the deviations in ln(*k*) from expected values (Figure 5b).

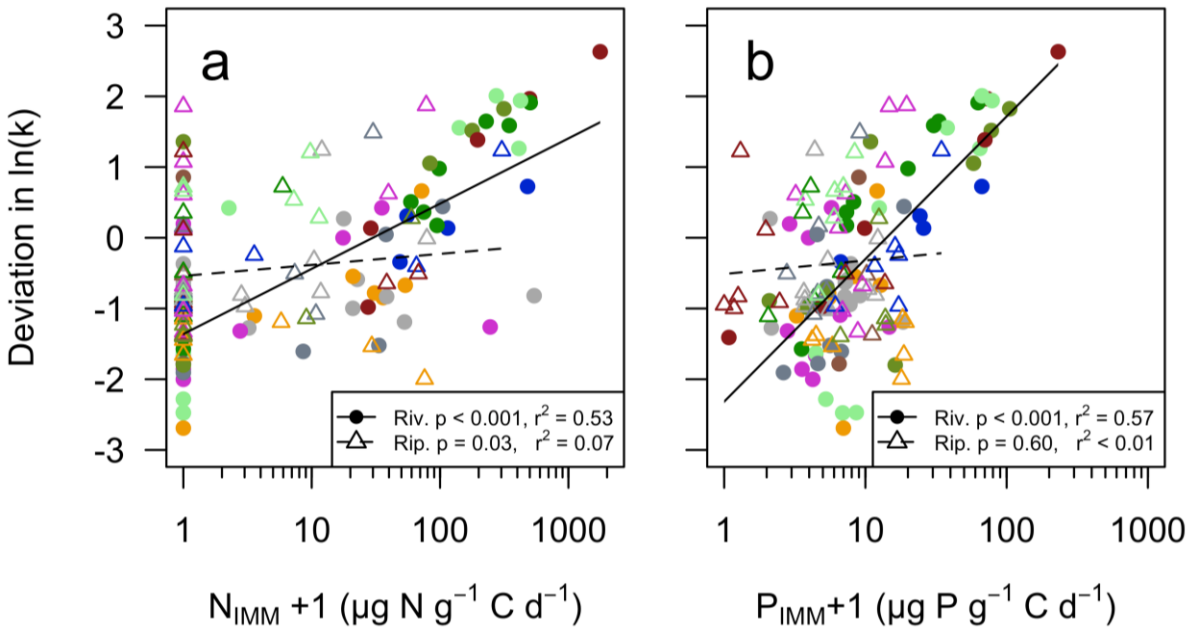

Figure 5. Relationship between nitrogen (a) and phosphorus (b) immobilization rates on cotton and deviations in decomposition rate from global trends in temperature sensitivity (Tiegs et al. 2019). In rivers, faster rates of nutrient immobilization are related to positive deviation (i.e., decomposition rates that are faster than the global average at that temperature). Nutrient immobilization on cotton in riparian soils was not predictive of deviations in decomposition.

4 Discussion

The microbes on our experimental cotton substrates immobilized N and P during decomposition at rates within the range measured for natural leaf litter. In riparian zones, N_{IMM} on cotton was similar to rates measured on six leaf litter species (20–33 µg N g^{-1} C d^{-1}; Aber & Melillo, 1982) but lower than those for crop residues in agricultural soils (462 µg N g^{-1} C d^{-1}; Recous et al., 1995). In rivers, N_{IMM} on cotton was within the range of immobilization on natural litter measured either by N accumulation in residual litter (Robbins et al., 2019) or isotopic dilution in microbial pools (Cheever et al., 2013; Pastor et al., 2014). P immobilization on decomposing litter is rarely measured, but our rates of P_{IMM} on cotton were similar to those recorded for three litter types in an artificial stream (0–12 µg P g^{-1} C d^{-1}; Robbins et al., 2019). Our approach, like that used by most previous studies of nutrient immobilization, could not distinguish assimilation by autotrophs on cotton from immobilization by decomposers, but natural shading of the stream channel likely limited algal contributions to net nutrient uptake (Elosegi et al. 2018; Halvorson et al. 2019).

Nutrient immobilization exhibited broad spatial patterns for cellulose decomposing in riparian zones, whereas in rivers, rates were more strongly controlled by local factors. Terrestrial biome, which is a proxy for climate, offered limited explanatory power of immobilization for rivers (13–19%), but explained slightly more variance for immobilization in riparian zones (23–30%). Similarly, latitude was a poor predictor of immobilization with the exception of P_{IMM} in riparian zones, which was slower at high latitudes. We observed an unbalanced influence of temperature and moisture across the riparian–river boundary. Annual precipitation is understandably less of a driver in rivers given that the sites studied were perennial. Conversely, moisture likely influences microbial decomposition and immobilization in riparian zones given their drier environment

(Sutfin et al., 2016). Temperature also had greater influence on immobilization in riparian zones than rivers, likely due to differences in the accessibility of exogenous nutrients between habitats. Rivers provide a continuous supply of inorganic nutrients that are accessible to microbial decomposers, and the supply rates influence immobilization. However, riparian zones receive more irregular supply of nutrients, and microbes are reliant on biologically mediated processes (e.g., N fixation, extracellular enzymes) (Allison & Vitousek, 2005; Vitousek & Hobbie, 2000). Conductivity, pH, DOC, and ammonium differed in rivers within some biomes, but none of these variables corresponded with immobilization rates. Although there was a strong latitudinal gradient in PO_4^{3-} in our study sites, there was only a weak, but directionally consistent, negative relationship between latitude and P_{IMM} ($r^2 = 0.03$). Collectively, these results indicate that controls of nutrient immobilization on decomposing litter can differ profoundly between riparian zones and adjacent rivers.

We observed strong relationships between rates of cellulose degradation and P_{IMM}, which suggests that P availability is a fundamental control on microbial decomposition of low-nutrient plant litter. Although we did not directly manipulate nutrients, our findings are consistent with experiments that have demonstrated faster decomposition under nutrient enrichment in many biomes (Ferreira et al., 2015). Correlations between immobilization and decomposition were stronger for P than N in both rivers and riparian zones. Enrichment of N and P have both been shown to stimulate microbial decomposers (Ferreira et al., 2015; Rosemond et al., 2015; Woodward et al., 2012), but in some rivers only P enrichment has stimulated decomposition (Burdon et al., 2020; Elwood et al., 1981; Newbold et al., 1983). The substrate used in this study was low in both N and P, but the relative abundance of these two elements (N:P = 62:1) indicated a greater P imbalance relative to microbial decomposers. This relative N enrichment is

common for litter; global analyses indicated average litter N:P of 46:1 for all senesced leaves (McGroddy et al., 2004) and 58:1 for riparian litter (Boyero et al., 2017). Therefore, P-limited litter may be a common substrate for microbial decomposers regardless of streamwater nutrient concentrations, and immobilization of exogenous P may be a critical limiting reaction for decomposition. Human-dominated catchments often receive excessive inputs of P from fertilizers or sewage (Birk et al., 2020; Carpenter et al., 1998), and our study provides further evidence that nutrient loading may increase organic matter decomposition in addition to its widely recognized enhancement of plant growth.

The relationships we observed between immobilization rates and water quality demonstrate the importance of exogenous sources of N and P for microbial decomposers in rivers globally (Woodward et al., 2012). The strong positive relationship between PO_4^{3-} and P_{IMM} is reasonable given the P-limited substrate. However, future work would be beneficial to understand the relative contribution of heterotrophic assimilation, autotrophic assimilation, and abiotic precipitation of P (potentially stimulated by microbial activity) to our calculated immobilization rates. The lack of relationship between dissolved N and N_{IMM} may be explained by the microbial decomposers being P limited or due to alternative sources of N. We observed river sites with relatively high DIN (>200 µg L^{-1}) that immobilized no N during decomposition. In these rivers, P concentrations were low (PO_4^{3-} <5 µg L^{-1}) and the substrate may have supplied sufficient N to support microbial activity. Alternatively, some rivers had relatively low DIN concentrations (<10 µg L^{-1}) with high rates of N_{IMM}, which may have been sustained by organic sources of N and/or atmospheric N_2 through N fixation. Finally, the cotton substrate is a potential carbon source for denitrifying bacteria, whose dissimilatory N products (i.e., N gasses) would not be included in N_{IMM}. However, the thin woven structure of the cotton strips and the deployment locations (i.e.,

in the water column and at the soil surface) would be unlikely to generate the anoxic sites needed for denitrification.

Our data indicate that the stoichiometry of microbial immobilization is influenced by microbial biomass N:P and not availability in exogenous pools or substrate imbalance. When microbes were immobilizing both nutrients, more N was immobilized than P (all N_{IMM}:P_{IMM} > 1), and the ratio of immobilized nutrients was constrained to a relatively narrow range (mean 10:1) among biomes and between rivers and riparian zones. On average, N_{IMM}:P_{IMM} was similar to the global mean N:P of microbial biomass in soil (7:1, Cleveland & Liptzin, 2007) and litter-associated fungal biomass (9:1, Gulis et al., 2017), suggesting the stoichiometry of heterotrophic immobilization is connected to the stoichiometry of microbial biomass. The mean N_{IMM}:P_{IMM} matched the global average for microbial biomass despite the fact that the litter was more deficient in P than N and many rivers provisioned nutrients at a low N:P (a third of rivers had DIN:PO_4^{3-} <10:1). Exogenous N:P in rivers varied widely (DIN:PO_4^{3-} between 1:1 and 1000:1), but N_{IMM}:P_{IMM} covered a much narrower range. Although bacterial and fungal taxa can be non-homeostatic (Danger & Chauvet, 2013; Godwin & Cotner, 2015) the communities decomposing cotton immobilized nutrients at a relatively fixed N:P across our globally distributed sites. This invariance in N:P at the ecosystem scale is consistent with observations of "ecosystem homeostasis" in bacterial-dominated heterotrophic rivers (Schade et al., 2011) and fixed ratios of exoenzymes in heterotrophic microbes (Hill et al., 2012; Sinsabaugh et al., 2009). Furthermore, the observed N_{IMM}:P_{IMM} may represent the averaging of the N:P of the microbial communities that can decompose this cellulose substrate (sensu Klausmeier et al., 2004). Consequently, if

microbes are P-limited, increasing PO_4^{3-} through nutrient loading is expected to increase rates of P_{IMM} and N_{IMM}, as microbes immobilize nutrients at a fixed ratio.

Although immobilization in rivers was not directly influenced by surface water temperatures, about half of the previously observed residual variation in the temperature dependence of decomposition (Tiegs et al., 2019) was explained by N_{IMM} and P_{IMM}. Thus, while water temperature sets the potential rate of decomposition, the external nutrient supply appears to determine whether microbes achieve the maximum potential rate. In contrast, riparian nutrient immobilization was directly controlled by temperature and thus co-varied with decomposition. Together these observations suggest that warmer temperatures in the future may have contrasting effects on decomposition and immobilization in riparian zones versus rivers. Although terrestrial decomposition is expected to increase with warming, our data indicate that future changes in precipitation may have more of an influence on riparian immobilization and decomposition than warming (Tiegs et al., 2019; Yin et al., 2019). Decomposition was temperature-sensitive in rivers, which supports predictions of faster decomposition under warming conditions (e.g., Boyero et al., 2011; Follstad Shah et al., 2017; Tiegs et al., 2019), but immobilization did not show strong relationships with temperature. Therefore, warming may not increase decomposition rates if surface water nutrient supply is stable or declining. The interactive effects of warming and eutrophication are well-recognized in autotrophic ecosystems (e.g., Binzer et al., 2016), and here we identified pathways by which these two global stressors may interact in heterotrophic ecosystems.

In rivers, leaf litter delivers an important subsidy of C to fuel microbial decomposers, but the N and P in microbial biomass is derived from both the plant litter itself and the environment. To understand the relative contributions of these two processes we made a hypothetical comparison

513 of N and P fluxes that were sourced exclusively from plant litter or solely from the environment
514 (i.e., litter only provides C). For representative ecosystems within each biome (Table S2), we
515 compared expected annual nutrient flux from leaf litter tissue (using models from Boyero et al.,
516 2017) to the flux from nutrient immobilization (using mean P_{IMM}, N_{IMM}, and T_{IMM} for each
517 biome). Across all biomes, leaf litter contributed more N to rivers than microbial immobilization
518 (Figure 6a). At most, immobilized N flux was 60% of the flux expected from leaf litter (in
519 tropical dry and temperate broadleaf forests). For P, it is possible for immobilization to provide a
520 similar or larger annual flux of P to microbial pools than leaf litter (Figure 6b), with larger
521 immobilization fluxes than litter flux possible in deserts and temperate broadleaf forests.
522 Microbial N and P on natural litter is a mix of litter-derived and immobilized nutrients, but this
523 hypothetical analysis demonstrates that the two sources may be comparable, especially for P.
524 However, nutrients supplied through litter may be in relatively recalcitrant organic molecules,
525 whereas immobilized N and P are likely to be in more labile forms. The eventual fate of
526 endogenous and exogenous nutrients involved in litter breakdown has consequences for the long-
527 term fate of nutrients, whether those detrital nutrients fuel riverine communities or are
528 transported downstream.

529

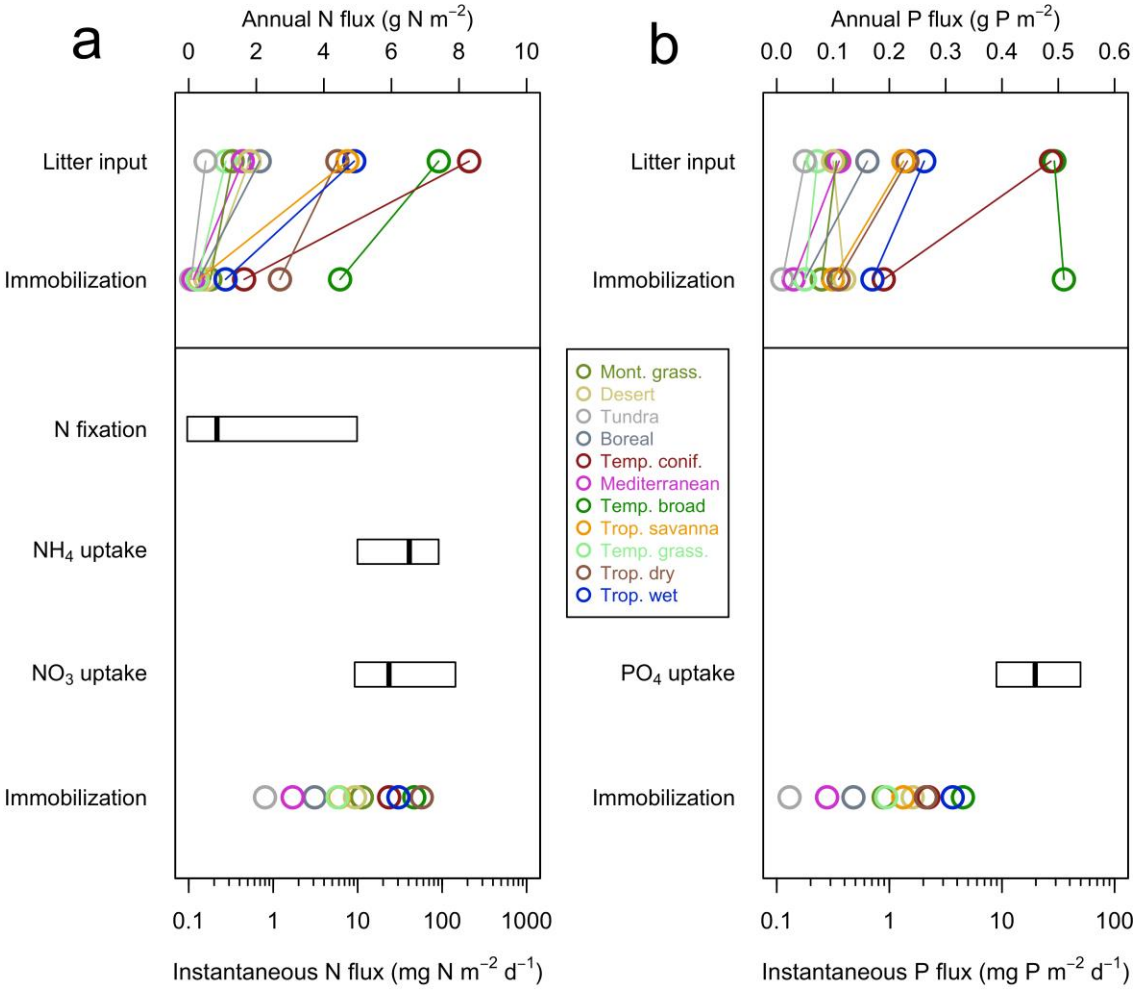

Figure 6. Nitrogen (a) and phosphorus (b) immobilization areal flux in rivers compared to other major fluxes. Immobilization fluxes are the product of geometric mean rates for a biome and measured litter fluxes in representative rivers within those biomes (see Table S2 for site details). "Litter input" shows N and P flux from endogenous nutrients in leaf litter; estimates are the product of litter flux in representative rivers and N and P content, predicted from mean annual precipitation (N) and mean annual temperature (P) (Boyero et al. 2017). Instantaneous N and P flux assume a concentrated pulse of leaf litter (i.e., time of litter fall << time of immobilization) and rates of litter input from representative rivers. NO_3^-, NH_4^+, and PO_4^{3-} uptake are whole-stream uptake (U) summarized by boxplots of median (vertical line) and interquartile range (IQR). Measurement of NH_4^+ and PO_4^{3-} uptake are from the review by Ensign and Doyle (2006), and measurement of NO_3^- uptake is from Ensign and Doyle (2006) and the LINX-II study (Hall et al. 2009). N fixation fluxes are summarized as a median and IQR from Marcarelli et al. (2008).

We also examined whether immobilization can account for a large portion of nutrient flux at the ecosystem scale by comparing microbial immobilization fluxes to whole-stream nutrient fluxes. The flux of nutrients due to immobilization only occurs during the seasons of peak litter fall (i.e., autumn in temperate zones and the dry season in the tropics) and instantaneous rates of immobilization were comparable to whole-stream uptake for N but not P. Instantaneous N immobilization was highest in forest biomes and was similar or higher than whole-stream uptake of NH_4^+ and NO_3^- in many biomes (Figure 6a). Instantaneous N immobilization was higher than N fixation by 1–2 orders of magnitude (Figure 6a). Thus, during peak litterfall, immobilization by microbes may be the dominant flux of N from the water column. Although our estimates showed that P flux from immobilization could be similar to P flux from litter, instantaneous P immobilization was 1–2 orders of magnitude lower than estimated whole-stream PO_4^{3-} uptake (Figure 6b). Riverbeds remove P through both biotic assimilation and abiotic sorption, and microbial immobilization only approaches this flux at very high rates of litter input (e.g., temperate broadleaf and tropical wet forests). For streams where both N and P uptake were measured, the average N:P ratio of whole-stream uptake (NH_4^+:PO_4^{3-} = 4:1, NO_3^-:PO_4^{3-} = 3:1, Ensign & Doyle, 2006) was lower than the average N:P of immobilization (10:1). Furthermore, whole stream uptake NO_3^-:PO_4^{3-} was <1 in 25% of streams (n = 65), but N_{IMM}:P_{IMM} <1 was never observed in our study. These comparative approaches indicate that the total flux of P from immobilization may be comparable to leaf litter inputs and N immobilization may be an important component of whole-stream uptake during peak litterfall.

Local variation in ecosystem characteristics can be as important as global scale factors when considering drivers of decomposition (Bradford et al., 2015, 2017). Most studies emphasize local-scale variation in litter quality as the primary control of decomposition (Bradford et al.,

2015; Follstad Shah et al., 2017; LeRoy et al., 2020); yet, we observed substantial local-scale variation in decomposition of a uniform substrate that we linked to differences in nutrient supply and temperature. Moreover, we observed substantial patch-scale differences in the controls and rates of microbial decomposition across river–riparian boundaries within ecosystems. By exploiting natural gradients in nutrient concentrations and climate at a global scale, we identified the direct and indirect pathways by which exogenous nutrient supply and temperature modify microbial processes. Although degradation of cotton strips surely differs from decomposition of plant litter, the broad mechanisms and patterns identified here would presumably apply to any organic substrate with low nutrient content. In particular, the stoichiometric constraints on immobilization in rivers across a broad gradient in nutrient supply and the temperature invariance of immobilization rates among globally distributed rivers are new insights about how nutrient loading and elevated temperatures from climate change may directly influence riverine decomposition.

Acknowledgements

We thank a large number of assistants who helped field work, G. Moeen and M. Moeen for preparing cotton strips, and N. Johnson, A. Minerovic, and C. Blackwood for help with C:N analysis. This research was supported by awards to S.D.T. from the Ecuadorian Ministry of Science (Secretaría de Educación Superior Ciencia, Tecnología e Innovación, SENESCYT) through the PROMETEO scholar-exchange program, the Oakland University Research Development Grant program, and a Huron Mountain Wildlife Foundation research grant. N.A.G. was supported by the U.S. Department of Energy's Office of Science, Biological and Environmental Research. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725. PRL was supported by

NSERC and Canada Research Chair programs. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Data Availability Statement

All data and code for analyses and figures are available on GitHub which can be accessed from the persistent DOI https://doi.org/10.5281/zenodo.5764917.

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