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Experimental nitrogen and phosphorus additions increase rates of stream ecosystem respiration and carbon loss

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Abstract

Nitrogen (N) and phosphorus (P) enrichment reduces organic carbon (C) storage in detritus-based stream ecosystems, but the relative effects of N and P concentrations and ratios on stream metabolic rates have not previously been tested. We tracked changes in whole-stream ecosystem respiration (ER) and gross primary productivity (GPP), particulate organic matter (POM) standing stocks, fungal biomass, and POM-specific respiration rates before and during 2 yr of experimental N and P enrichment in five forest streams. Nutrient additions ($\sim$ 96 μg N L^{-1} to $\sim$ 472 μg N L^{-1} and $\sim$ 10 μg P L^{-1} to $\sim$ 85 μg P L^{-1}) targeted dissolved N : P molar ratios of 2, 8, 16, 32, and 128. Whole-stream ER was positively related to standing stock of wood, a seasonably stable POM compartment that varied by up to 2$\times$ among streams. Nutrient enrichment generally increased ER but had no effect on low-level GPP. Prior to nutrient enrichment, ER was higher at lower N : P, but during enrichment ER increased with increasing N : P. Respiration rates on leaf litter and wood increased with enrichment but decreased with increasing P, and the quantity of leaf litter generally declined with increasing N. Respiration rates on fine benthic organic matter (FBOM) were higher with increasing N : P, and FBOM standing stocks decreased with increasing N. Fungal biomass did not change with nutrient enrichment. Compared to pre-enrichment conditions, nutrients increased seasonal variation in leaf litter standing stocks and whole-stream respiration rates. Our results demonstrate how nutrient-stimulated loss of C from detritus-based ecosystems occurs through the maintenance of enhanced respiration rates on detrital resources that are particularly sensitive to N inputs.

Ecosystem metabolism considers the balance of gross primary productivity (GPP) and ecosystem respiration (ER) at whole-ecosystem scales (Tank et al. 2010). Nutrient loading (i.e., nitrogen [N] and phosphorus [P]) from anthropogenic sources changes ecosystem metabolism either by increasing GPP and autotrophic respiration (i.e., more organic C fixed and respired by autotrophs), or by increasing ER via a greater heterotrophic response that reduces organic C storage (Peterson et al. 1985; Gulis and Suberkropp 2003; Gulis et al. 2004). Although increases in GPP and autotrophic respiration are generally predicted with N and P addition (Dodds et al. 2002; Elser et al. 2007), nutrient effects on heterotrophic respiration are less studied. A multi-site, cross-biome comparison of different drivers of whole-stream GPP and ER found consistent effects of elevated nutrients and light on GPP, and of particulate organic matter (POM) quantity mediated by nutrients on ER (Bernot et al. 2010). Experiments that test nutrient effects on the heterotrophic component of ecosystem metabolism are needed to provide a clearer understanding of the relative effects of N and P pollution on C processing in streams.

Many inland waters, including streams, respire more C than they fix because they receive inputs of allochthonous POM that is subsequently broken down in situ by decomposer communities (Webster et al. 1999). Responses of heterotrophic respiration to nutrient enrichment are likely driven by several factors, including nutrient concentration, C inputs and pools, and microbial community activity

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(Dodds 2007). Previous work by our research group has showed that increased nutrient concentrations reduce detritus storage in streams via enhanced microbial and invertebrate processing (Benstead et al. 2009; Suberkropp et al. 2010; Rosemond et al. 2015; Manning et al. 2016). Carbon residence times are reduced with added N and P (Rosemond et al. 2015), with more detrital C predicted to be respired closer to its location of input, reducing retention, and downstream export. Nutrients consequently alter both storage (Benstead et al. 2009) and area-specific respiration of detrital C (Suberkropp et al. 2010), as temporally dynamic inputs (e.g., autumn-shed leaf litter) are more rapidly processed in situ. Added nutrients are expected to have these effects via the release of heterotrophs from nutrient limitation, unless respiration becomes seasonally limited by C availability (e.g., Valett et al. 2008; Huryn et al. 2014). Collectively, these predictions of higher total annual respiration near the location of C inputs, as well as higher seasonal variation in area-specific respiration of detrital C, require testing at ecosystem scales.

Reductions in detrital POM, along with substrate-specific increases in microbial respiration (R), have been shown after low to moderate experimental increases in N and P (Benstead et al. 2009; Suberkropp et al. 2010; Rosemond et al. 2015). However, the consequences of added N and P and their relative limiting effects on whole-stream ER, which incorporates both the quantity of substrates and activity of decomposers, have not been previously tested. Further, effects of experimental N and/or P addition have largely been tested at single nutrient concentrations and N : P ratios in individual ecosystems (Gulis and Suberkropp 2003; Slavik et al. 2004; Benstead et al. 2009; Deegan et al. 2012). As dissolved N and P concentrations do not necessarily co-vary across land-use gradients (e.g., Taylor et al. 2014), understanding the relative importance and interactive effects of N and P on ecosystem metabolism and C storage requires testing at multiple ratios along the low-to-moderate gradients in N and P that are now common across landscapes (Kominoski et al. 2015; Manning et al. 2015; Rosemond et al. 2015).

Here, we test how N and P enrichment alters detrital POM standing stocks and whole-stream ecosystem metabolism (GPP and ER) using experimental N and P additions to five forest streams. We chose dissolved nutrient concentrations and ratios that reflected a range of landscape conditions, including relatively pristine to moderately impacted streams (Alexander and Smith 2006; Woodward et al. 2012). We expected added N and P to increase GPP during periods of higher temperature and light availability (i.e., before leaf-out in spring). We predicted that higher N and P would increase ER during periods of high detritus availability (i.e., after leaf-fall in autumn), driven by stimulation of heterotrophic processing of detrital POM, possibly resulting in higher annual ER rates (Fig. 1). We also predicted subsequent declines in detrital POM standing stocks with added N and P

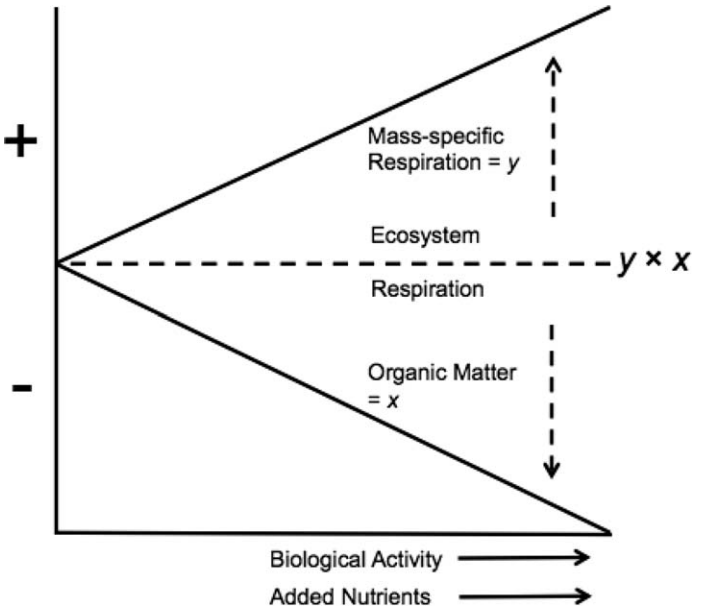

Fig. 1. Conceptual model predicting effects of increased biological activity and added nutrients on detrital POM standing stocks (x, lower line), mass-specific respiration (y, upper line), and their product (ER, dashed line).

(Benstead et al. 2009; Suberkropp et al. 2010), leading to eventual decreases in daily whole-stream ER and increases in seasonal variation of ER rates. We expected whole-stream ER to be explained by substrate-specific R and fungal biomass scaled to whole streams based on POM standing stocks. Our treatment included enrichment of both N and P in all streams, but in opposing gradients (high N, with low P; high P with low N), which allowed us to test the relative importance of N and P at established ratios. We predicted enhanced responses to added N and P across N : P ratios, consistent with findings of N and P co-limitation in these detritus-based ecosystems (Ferreira et al. 2015; Kominoski et al. 2015; Rosemond et al. 2015).

Methods

Site description and experimental design

We studied 70-m reaches of five first-order streams in the Dryman Fork catchment at Coweeta Hydrologic Laboratory, a USDA Forest Service research station and Long Term Ecological Research (LTER) site in the southern Appalachian Mountains in Macon County, North Carolina, U.S.A (Swank and Crossley 1988). Following a year of pretreatment (PRE) data collection, we began continuously dosing the entire length of each 70-m reach on 11 July 2011 with concentrated solutions of ammonium nitrate (NH_4NO_3) and phosphoric acid (H_3PO_4) using solar-powered metering pumps (LMI Milton Roy, Ivyland, Pennsylvania, U.S.A.) connected to gravity-fed irrigation lines supplied with stream water. Nutrient dosing in each stream was proportional to stream

discharge, which was estimated based on stage-discharge rating curves for each stream. We measured water velocity and discharge ($n = 24$ measurements from each of the five streams) via dilution gaging using salt (NaCl; Gordon et al. 2004). We measured stage continuously with pressure transducers (Keller America, Newport News, Virginia, U.S.A.) and nutrients were dispensed based on cumulative discharge records from CR-800 dataloggers (Campbell Scientific, Logan, Utah, U.S.A.). Dripper spouts were placed ~ 5 m apart along the 70-m reach to ensure adequate mixing and compensation for uptake.

For 2 yr (YR1, YR2), each stream reach received a different concentrated solution of N and P to target five increasing concentrations of N (added + background = 81 μg L^{-1}, 244 μg L^{-1}, 365 μg L^{-1}, 488 μg L^{-1}, 650 μg L^{-1} as dissolved inorganic nitrogen [DIN]) and corresponding decreasing concentrations of P (added + background = 90 μg L^{-1}, 68 μg L^{-1}, 51 μg L^{-1}, 33 μg L^{-1}, and 11 μg L^{-1} as soluble reactive phosphorus [SRP]), resulting in a target N : P for each stream (2, 8, 16, 32, and 128, respectively). Multiple streamwater samples ($n = 4$; collected and analyzed as described below) were taken every ~ 15 m along each of the 70-m reaches on days 1, 4, 7, 14, 23, 29, and 34 of enrichment to confirm adequate mixing of added nutrients. After day 34 of enrichment, streamwater was collected above ($n = 1$) and below ($n = 3$, at 10-m, 17-m, and 70-m) the nutrient dosing system biweekly, filtered in the field (0.45-μm nitrocellulose membrane filters; Millipore, Billerica, Massachusetts, U.S.A.) and frozen until analyzed for DIN (NH_4-N + NO_3-N) and SRP concentrations (Alpkem Rapid Flow Analyzer 300 for DIN, spectrophotometric method with Shimadzu UV-1700 for SRP). Further details about the experimental design, infrastructure, and stream physicochemical characteristics can be found in Rosemond et al. (2015) and Manning et al. (2015).

Whole-stream ecosystem metabolism

Daily ecosystem metabolism parameters (GPP and ER) were estimated from open-channel dissolved oxygen (DO; YSI ProODO sondes, Yellow Springs, Ohio, U.S.A.), water depth, temperature, and light. Temperature was measured every 15 min continuously in each stream during PRE, YR1, and YR2 using submersible temperature dataloggers (Onset Computer Corporation, Pocasset, Massachusetts, U.S.A.). Photosynthetically active radiation (PAR; μmol s^{-1} m^{-2}) was measured continuously and averaged every 15 min at the upstream location of the metering pumps in each stream using a LI-193 spherical quantum sensor (LI-COR, Lincoln, Nebraska, U.S.A.) connected to a CR-800 datalogger. Monthly PAR measurements were also taken every 10 m in each stream to calculate daily light integration (DLI) averaged for each 70-m treatment reach from built relationships between monthly measurements integrating the stream reach and continuous measurements recorded at a single location upstream. Daily PAR was converted to units of mol m^{-2} d^{-1}. We calibrated oxygen sensors in air-saturated water by placing them in a bucket of aerated water for 20 min immediately prior to and following deployment to ensure consistent measurement of DO and temperature and to assess potential drift during deployment. DO in each treatment stream was recorded for 24–48 h (3-min interval) monthly (April–October) in PRE, YR1, and YR2 and continuously in YR2 toward the end of the enrichment experiment (June–July 2013) using a single DO sonde placed at the downstream end of each stream reach. We were unable to detect metabolic changes in O_2 in the winter (November–March) due to supersaturated DO and high reaeration rates ($K_{O2} > 0.3$ g O_2 m^{-2} min^{-1}). Oxygen flux was estimated by fitting the following model to the oxygen data (Van de Bogert et al. 2007; Hall et al. 2015):

$$O_i = O_{i-t} + \frac{GPP \times PPFD_t}{z \times \sum PPFD} + \frac{ER \times \Delta t}{z} + K(O_s - O) \times \Delta t, \quad (1)$$

where O_2 at time i is equal to O_2 at the previous time ($i-1$) plus time-step-specific rates of GPP and ER, z is water depth, and K is air–water gas exchange coefficient per unit time (based on the reaeration flux $K(O_s - O)$, and the difference between dissolved O_2 and O_2 at saturation for a given temperature and barometric pressure). O_s is saturated oxygen concentration, and $O_s - O$ is the saturation deficit. $PPFD_t$ is photon flux density during time period during the time interval (μmol photons m^{-2} s^{-1}). In this model, ER is a negative O_2 flux because O_2 is being consumed. The time-step (Δt) is the measurement interval of logged O_2 data for a one-station metabolism model in streams. Given the dense canopy cover of these forested streams, we assumed that GPP was a linear function of light (Van de Bogert et al. 2007) and that ER was constant throughout the day.

We estimated reaeration coefficients for each stream normalized to a Schmidt number of 600 (K_{600}, min^{-1}) based on empirically measured rates of oxygen exchange using sulfur hexafluoride (SF_6), a tracer gas, and rhodamine as a conservative tracer, collected throughout the sampling season (April–October) in each stream (Supporting Information Table S1). We continuously pumped SF_6 and rhodamine 10 m upstream of each metabolism reach to ensure appropriate mixing. We measured streamwater fluorescence during addition of the rhodamine tracer compared to background fluorescence (prior to rhodamine addition) at the downstream of the treatment reach using a hand-held fluorometer (Turner Designs, California, U.S.A.). We determined that a plateau had been achieved when fluorescence readings had stabilized for 15 min. We then collected duplicate 12-mL water samples (Exetainer vials, Labco, Ceredigion, UK) every 10 m along the 70-m treatment reach of all streams, using the rhodamine tracer to account for downstream dilution (Wanninkhof et al. 1990; Marzolf et al. 1994). Measured rates of oxygen exchange ($n = 11$ measurements from each of the

five streams) fit an exponential function with discharge in each stream (Supporting Information Fig. S1, R^2 range: 0.31–0.66), so reaeration rates were estimated from discharge (within the same range of measured reaeration) whenever direct measurements were unable to be made (e.g., during continuous measurements of metabolism; Roberts et al. 2007).

To estimate GPP and ER, we fit the model of diel whole-stream metabolism to the measured oxygen data by finding estimates of GPP and ER that minimized the negative log likelihood of the model to the data using function *nlm*() in R (Hall et al. 2015). We compared model fits based on negative log likelihood estimate (LLE), and LLEs > -500 were eliminated. These poor model fits to the data accounted for 12% ($n = 40$ of 322) of our daily estimates of GPP and ER, leaving $n = 282$ estimates. Estimates of GPP that were negative were sourced to high discharge that increased DO above levels attributed to photosynthesis (Hall et al. 2015); those values were determined to be non-detectable and converted to zero (51%, $n = 143$ of 282). Estimates of ER that were positive were sourced to probe calibration error; those estimates of ER were also eliminated ($n = 30$ of 282, or 11% of data).

Substrate-specific microbial respiration rates and fungal biomass

Substrate-specific respiration rates were measured as oxygen uptake of decomposing detrital POM at stream temperatures (see below and Gulis and Suberkropp 2003). Samples ($n = 4$) of fine benthic organic matter (FBOM), leaf litter, and wood were collected from streams quarterly (winter values not used) and placed in filtered stream water specific to each stream in darkened respiration chambers (30 mL for leaf litter and wood, 300 mL bottles for FBOM) in an incubator at mean stream temperature within 24 h of sample collection. DO concentrations were recorded every 5 min for 30 min in continuously stirred water (except for FBOM, whereby initial and final DO were recorded before and after 30 min incubations using water that was stirred for 30 s during readings) using YSI 5100 DO meters (Yellow Springs, Ohio, U.S.A.). Additional chambers ($n = 2$ per stream) containing only stream water specific to each stream served as controls. Oxygen consumption was determined as the slope of the regression of DO concentration over time minus the slope of the control, and respiration rates were expressed per gram ash-free dry mass (AFDM) per hour. Mass-specific microbial respiration rates were scaled to whole streams by multiplying by respective detrital POM standing stocks (see below: Detrital POM standing stocks) and summing up; values were expressed on an areal basis as g O_2 m^{-2} d^{-1}.

Frozen samples of leaf litter and wood were freeze-dried and weighed prior to estimation of mass-specific fungal biomass based on ergosterol concentrations. Lipids were extracted and ergosterol quantified by HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a Phenomenex Kinetex C18 column and a UV detector set at 282 nm (Newell et al. 1988; as modified by Gulis et al. 2006). To convert ergosterol concentration to fungal biomass, we assumed an ergosterol concentration of 5.5 μg mg^{-1} of mycelial dry mass (Gessner and Chauvet 1993). Whole-stream fungal biomass on an areal basis was estimated taking into account leaf litter and wood standing stocks. Wood-associated fungal biomass was normalized by wood diameter based on previous estimates from Coweeta, as larger pieces of wood have lower concentrations of fungal biomass than smaller pieces (Gulis et al. 2008).

Detrital POM standing stocks

We measured whole-stream FBOM and leaf litter standing stocks during PRE, YR1, and YR2 in each stream. Samples of FBOM were collected monthly using a benthic stovepipe core sampler. Four cores were taken at random locations within the 70-m experimental reach of each stream each month. Samples were processed according to Lugthart and Wallace (1992). An aliquot ($\sim$ 250 mL) of elutriated interstitial water was rinsed through nested soil sieves (1-mm and 250-μm mesh sizes); material retained on the 250-μm sieve was deemed to be FBOM and associated inorganic material. A subsample of the retained material was filtered onto a pre-ashed and weighed glass fiber filter that was dried at 60°C for at least 24 h and reweighed. Filters were combusted in a muffle furnace at 500°C for 3 h. Upon removal from the furnace, filters were rewetted and returned to the drying oven for an additional 24 h before being reweighed to obtain AFDM.

Leaf litter was collected monthly using a sampling quadrat (0.15-m width) across the wetted stream width at eight randomly selected transects along the 70-m study reaches as in Suberkropp et al. (2010). Leaf litter from transects was weighed, subsampled, oven-dried (60°C) for 48 h, weighed, combusted (550°C for 4 h), and re-weighed to determine AFDM on an areal basis (g AFDM m^{-2}). We also estimated reach-scale wood standing stocks in each stream using the line-intersect technique (Wallace and Benke 1984). Wood diameters (up to 10 cm) were measured to the nearest 0.1 cm every 5 m using Vernier calipers within the wetted area of the stream ($n = 15$; Wallace and Benke 1984). Volume was computed for each unique diameter measurement, converted to g AFDM based on a previously reported specific gravity conversion factor (Wallace et al. 2001) and then summed for each transect. As with leaf litter, wood standing stocks are reported on an areal basis (i.e., g AFDM m^{-2}). As wood standing stocks are relatively stable from year-to-year in these streams (Webster et al. 1999; Wallace et al. 2001), our one-time measurement of wood reflects standing stocks throughout our study.

Data analyses

We used the pre-enrichment year as a baseline control in all five experimental streams. Although a lack of temporal

Table 1. Physicochemical characteristics in streams ($n = 5$) during the sampling periods (April–October) before (PRE) and during nutrient enrichment (YR1, YR2). Mean discharge (± SE), mean temperature (range), and mean DLI (range) are the daily averages measured on days when ecosystem metabolism was estimated. Nutrient concentrations (DIN; SRP) reported are targeted and measured (biweekly) average (± SE) concentrations (μg L^{-1}) and molar ratios for each stream based on biweekly measurements.

Stream targeted DIN, SRP, N : P	Location	Year	Mean daily discharge (L s^{-1})	Mean daily temperature (°C)	Mean DLI (mol m^{-2} d^{-1})	Mean monthly DIN (μg L^{-1})	Mean monthly SRP (μg L^{-1})	Mean monthly N : P
81, 90, 2	35°01′49″N, −83°27′06″W	PRE	5.2 ± 1.1	11.6 (9.2–15.6)	2.0 (0.2–3.7)	12.0 ± 0.0	4.2 ± 0.0	6
		1	1.5 ± 0.5	13.5 (12.3–14.3)	0.8 (0.1–1.6)	49.9 ± 11.5	22.1 ± 2.1	5
		2	5.8 ± 0.7	14.1 (9.7–16.8)	0.4 (0.2–3.1)	58.5 ± 2.3	56.5 ± 1.7	2
244, 68, 8	35°01′51″N, −83°27′06″W	PRE	20.1 ± 2.9	12.3 (9.1–15.1)	1.6 (0.6–2.7)	112.2 ± 21.1	3.4 ± 0.3	73
		1	12.2 ± 1.3	12.8 (11.6–15.8)	0.7 (0.2–1.3)	193.7 ± 34.2	75.2 ± 20.3	6
		2	15.6 ± 1.8	12.5 (9.8–14.4)	0.7 (0.1–1.8)	132.2 ± 1.8	25.8 ± 1.5	11
365, 51, 16	35°01′40″N, −83°27′08″W	PRE	5.0 ± 1.4	11.9 (9.1–14.8)	1.2 (0.3–1.8)	16.0 ± 0.0	6.6 ± 0.0	5
		1	4.3 ± 0.5	13.2 (11.5–16.5)	0.6 (0.2–1.1)	341.8 ± 98.3	37.6 ± 6.7	20
		2	6.8 ± 0.5	13.9 (11.3–14.5)	1.2 (0.3–3.1)	163.2 ± 9.3	23.1 ± 0.1	16
488, 33, 32	35°01′25″N, −83°26′58″W	PRE	10.6 ± 2.5	12.9 (9.3–16.3)	3.2 (0.6–10.1)	183.5 ± 4.6	4.0 ± 0.5	102
		1	4.3 ± 0.2	13.6 (12.1–17.7)	2.2 (0.1–7.2)	435.8 ± 122.3	27.4 ± 5.4	35
		2	5.8 ± 0.3	13.1 (11.5–15.9)	2.1 (1.0–2.5)	194.4 ± 2.7	12.6 ± 0.2	34
650, 11, 128	35°01′37″N, −83°27′05″W	PRE	6.9 ± 2.3	14.1 (9.2–16.6)	2.07 (0.5–5.4)	38.1 ± 1.1	3.0 ± 0.3	28
		1	3.8 ± 0.6	13.7 (11.5–18.1)	1.2 (0.3–1.8)	380.7 ± 119.3	8.4 ± 2.2	100
		2	7.0 ± 0.4	13.8 (12.8–16.3)	0.2 (0.1–0.5)	139.5 ± 14.16	5.4 ± 0.5	57

replication of our controls for each stream violates assumptions of inferential statistics (Hurlbert 1984), the multiple catchment, ecosystem-scale of our experiment, in addition to the multiple years of data acquired, were essential for understanding ecosystem-level responses to differences in added N and P concentrations and ratios. To analyze our data, we developed hierarchical linear mixed-effects models using the R package "lme4" (Bates et al. 2015). Models with continuous, fixed effects of dissolved N and P concentrations (seasonal [except winter] averages from biweekly measurements) and measured N : P ratios (Table 1), categorical fixed effects of year (PRE, YR1, YR2), and random effects of stream nested in year tested for effects on whole-stream ER, whole-stream GPP, substrate-specific respiration rates associated with detrital POM (FBOM, leaf litter, wood), detrital POM standing stocks (FBOM, leaf litter), and temporal changes in leaf litter standing stocks. We excluded winter values of detrital POM standing stocks and substrate-specific respiration rates from linear mixed-effects models given that we were unable to collect whole-stream metabolism during winter (see above). We corrected microbial respiration rates associated with FBOM, leaf litter, and wood from the temperatures measured during incubations to corresponding mean stream temperature on the day detrital POM was sampled. Respiration rates were temperature-corrected following Brown et al. (2004):

$$R_s = R_i \times \frac{e^{-E/kT_s}}{e^{-E/kT_i}}, \quad (2)$$

where R_s and R_i are respiration rates at stream and incubation temperatures, respectively, E is the predicted activation energy (0.65 eV) associated with aerobic respiration, k is the Boltzmann constant (8.62×10^{-5}), and T_s and T_i are temperature in degrees Kelvin for the stream and the incubator, respectively.

All response and predictor variables were $\log_{10}$-transformed to meet assumptions of normality. We standardized continuous predictor variables using z-scores to compare variables measured at different scales, and to aid the interpretation among continuous predictors (i.e., N and P concentrations; Gelman and Hill 2007). Akaike's information criterion corrected for small sample size (AIC_C; Burnham and Anderson 2002) was calculated using the R package "AIC_Cmodavg" (Mazerolle 2013). Akaike model weights were used to estimate the likelihood that each factor was in the top model, and selection of the most parsimonious model was based on delta AIC_c (delta $AIC_c \leq 2$). Using the MuMIn package in R (Bartoń 2016), we assessed goodness of fit

Table 2. Seasonal (except winter) estimates of whole-stream ecosystem metabolism from single-station, diel measurements of stream DO ($n = 252$) during PRE and enrichment years (YR1, YR2). Values for GPP and ER are seasonal means (SE) collected throughout April–October during each treatment year. Missing values are denoted with "-."

Stream targeted N : P	Year	GPP (g O_2 m^{-2} d^{-1})			ER (g O_2 m^{-2} d^{-1})		
		Spring	Summer	Autumn	Spring	Summer	Autumn
2	PRE	0.1 (0.1)	0.0	-	−4.5 (−3.2)	−2.2 (−2.2)	-
	1	0.0	0.0	0.0	−3.2 (−2.2)	−12.8 (−12.8)	−2.7 (−2.7)
	2	0.0	0.2 (0.0)	0.0	−1.0 (−1.0)	−16.3 (−3.5)	−2.7 (−1.9)
8	PRE	0.4 (0.3)	<0.1 (0.0)	0.0	−2.5 (−1.8)	−6.6 (−4.6)	−8.2 (−8.2)
	1	0.1 (0.1)	0.00	0.0	−4.5 (−3.2)	−2.4 (−2.1)	−8.2 (−5.8)
	2	0.3 (0.3)	0.1 (0.0)	<0.1 (0.0)	−0.7 (−0.7)	−6.4 (−1.0)	−1.4 (−1.0)
16	PRE	0.1 (0.1)	<0.1 (0.0)	-	−5.5 (−3.9)	−9.1 (−6.4)	-
	1	<0.1 (0.0)	0.0	0.2 (0.2)	−8.9 (−6.3)	−21.7 (−15.3)	−1.9 (−1.3)
	2	-	0.4 (0.1)	0.1 (0.1)	-	−12.4 (−1.9)	−12.3 (−12.3)
32	PRE	0.3 (0.2)	0.0	0.0	−0.6 (−0.4)	−3.3 (−2.4)	−0.7 (−0.7)
	1	<0.1 (0.0)	<0.1 (0.0)	0.0	−2.8 (−2.0)	−3.7 (−2.6)	−4.6 (−3.3)
	2	-	<0.1 (0.0)	0.0	-	−2.5 (−0.4)	−3.4 (−2.4)
128	PRE	0.4 (0.4)	<0.1(0.0)	<0.1 (0.0)	−5.0 (−5.0)	−6.4 (−4.5)	−1.8 (−1.8)
	1	0.1 (0.1)	0.00	<0.1 (0.0)	−6.3 (−6.3)	−5.2 (−3.7)	−5.7 (−4.0)
	2	-	0.2 (0.0)	0.0	-	−5.5 (−0.9)	−6.2 (−6.2)

(conditional R^2; Nakagawa and Schielzeth 2013) for top mixed-effects models to determine how well factors explained variance among response variables.

We compared whole-stream ER to gradients in dissolved N : P, leaf litter and FBOM standing stocks, wood standing stocks, and fungal biomass scaled to whole streams (based on leaf litter and wood standing stocks) in PRE, YR1, and YR2 using simple linear regression. We compared regression slopes using analysis of covariance (ANCOVA). We tested for effects on added N and P on substrate-specific R (leaf litter, FBOM, and wood) and fungal biomass associated with leaf litter and wood in PRE, YR1, and YR2 using ANOVA.

We compared intra-annual coefficients of variation for FBOM and leaf litter standing stocks, areal-specific fungal biomass, substrate-specific R scaled to whole streams, and whole-stream ER across the five streams using ANOVA. Orthogonal contrasts were specified a priori to test for differences among targeted dissolved N : P treatments (e.g., comparing targeted N : P treatments above and below 16:1). These contrasts were performed on whole-stream ER and ER normalized per g wood biomass to account for possible effects of differences in wood standing stocks on ER in the five streams. All statistical analyses were performed in R v. 3.0.1 and RStudio v. 0.89.501 (R Core Team 2013).

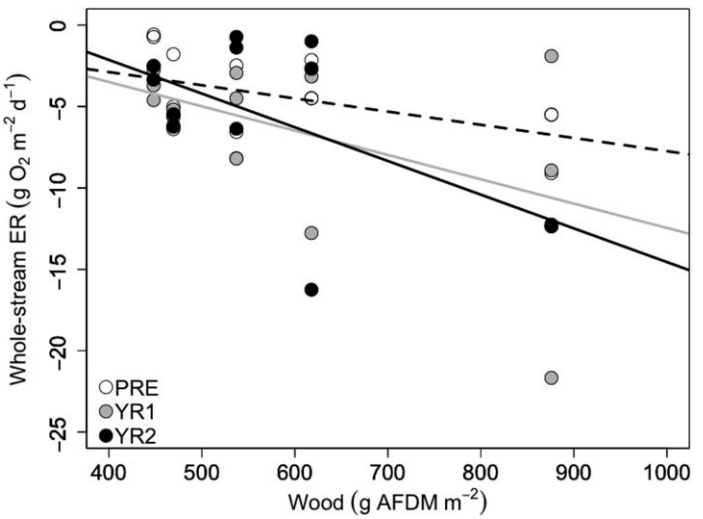

Fig. 2. Whole-stream ER estimated as seasonal (except winter) means of diel measurements of DO ($n = 252$) before (PRE) and during 2 yr (YR1, YR2) of experimental additions of DIN (N) and SRP (P). Open circles correspond to PRE conditions, filled gray and black circles correspond to YR1 and YR2 of enrichment, respectively. The dashed black line corresponds to PRE slopes, and the solid gray and black line corresponds to slopes for YR1 and YR2, respectively. Whole-stream ER along gradients in wood standing stocks (one-time estimate). Wood standing stocks were correlated with higher whole-stream ER in PRE ($y = -0.01x + 0.36$; Adj. $R^2 = 0.20$, $p = 0.05$) and YR2 ($y = -0.02x + 6.15$; Adj. $R^2 = 0.41$, $p < 0.01$), but not in YR1 ($y = -0.01x + 2.49$; Adj. $R^2 = 0.16$, $p = 0.08$). Slopes of the regression of whole-stream ER with wood standing stocks in PRE, YR1, and YR2 were tested with ANCOVA, indicating differences among years (Adj. $R^2 = 0.26$, $p < 0.01$). A negative slope of linear regressions of negative values of whole-stream ER indicates a positive (higher ER) response.

Results

Experimental conditions

Before enrichment, stream concentrations of DIN (range = 12–139 μg L^{-1}) were more variable among streams than those of SRP (range = 3–7 μg L^{-1}; Table 1). Nutrient

Table 3. Linear mixed-effects models and model weights of fixed effects of added nutrients [DIN (NO3-N + NH4-N; N) and SRP (P) concentrations (μg L^{-1})] and stream temperature, and random effects of stream and year within stream (Stream/Year) on seasonal (except winter) whole-stream ER and GPP, substrate-specific respiration rates (R) associated with detrital POM [FBOM, leaf litter, and wood], and leaf litter and FBOM standing stocks. Response variables and fixed predictor variables (N, P, and N : P) were first $\log_{10}$-transformed to meet assumptions of normality. Fixed predictor variables were standardized using z-scores. Categorical variables (e.g., stream, year) were not transformed. Seasonal (except winter) whole-stream ER was estimated from single-station, diel measurements of DO ($n = 252$) before and during N and P enrichment. Changes in leaf litter standing stocks, from the maximum to minimum quantity on the streambed, were calculated for each stream before and during each year of enrichment. Changes (Δ) in leaf litter standing stocks were tested using separate linear mixed-effects models (fixed effects of N, P, N : P; random effects of stream). Bolded values of conditional R^2 are significant ($p < 0.05$). Notes: Akaike's information criterion adjusted for small sample sizes (AICc) was used to identify model parsimony. The difference in AICc scores from the top model (lowest AICc) is ΔAICc. AICc wt is the weighted AICc score, which is calculated as ΣAICc/AICci. $AIC = 2K - 2\ln(L) + K$; $AICc = AIC_c + 2K(K+1)/(n-K-1)$, whereby K is the number of parameters in the model, L is the likelihood function for the model, and n is sample size. Cum wt is the cumulative model weights of evidence. Models with $\Delta AICc \leq 4$ are considered equivalent (Burnham and Anderson 2002).

Models	K	ΔAIC_c	AICc wt	Cum wt	Log likelihood	Equation and conditional R^2
Whole-stream ER (g O_2 m^{-2} d^{-1})						
N : P	5	0.00	0.34	0.34	−14.95	$y = -0.09$(N : P) + 0.62, **0.22**
N : P*year	7	0.23	0.30	0.64	−12.32	$y = -0.19$(N : P*PRE) + 0.01(N : P*YR1) + 0.12(N : P*YR2) + 0.65, **0.24**
P	5	1.75	0.14	0.78	−15.82	$y = 0.04$(P) + 0.62, **0.20**
Whole-stream GPP (g O_2 m^{-2} d^{-1})						
Year	6	0.00	0.52	0.52	4.17	$y = -0.15$(YR1) + 0.05(YR2) − 0.76, **0.14**
Substrate-specific R (g O_2 g AFDM^{-1} d^{-1})						
FBOM						
N : P	5	0.00	0.61	0.61	255.93	$y = 0.0003$(N : P) + 0.002, **0.15**
N : P*year	7	1.44	0.30	0.91	257.95	$y = 0.0005$(N : P*PRE) − 0.000003(N : P*YR1) + 0.0007(N : P*YR2) + 0.002, 0.07
Leaf litter						
P	5	0.00	0.43	0.43	214.88	$y = 0.001$(P) + 0.005, **0.18**
Year	6	1.57	0.19	0.62	215.43	$y = 0.002$(YR1) + 0.002(YR2) + 0.003, **0.20**
N	5	1.84	0.17	0.79	213.96	$y = 0.001$(P) + 0.005, **0.15**
P*year	7	1.86	0.17	0.96	216.96	$y = 0.001$(P*PRE) + 0.001(P*YR1) − 0.0002(P*YR2) + 0.005, **0.25**
Wood						
P*year	7	0.00	0.70	0.70	318.30	$y = 0.0002$(P*PRE) − 0.00004(P*YR1) + 0.0001(P*YR2) + 0.0004, **0.35**
Standing stocks (g AFDM m^{-2})						
FBOM						
N	5	0.00	0.46	0.46	−214.50	$y = -12.30$(N) + 73.50, **0.35**
N : P	5	0.95	0.28	0.74	−214.97	$y = -11.02$(N : P) + 73.50, **0.31**
Leaf litter						
Year	6	0.00	0.27	0.27	−326.84	$y = -255.63$(YR1) − 26.98(YR2) + 426.12, **0.10**
N*year	7	0.02	0.27	0.54	−325.44	$y = -111.22$(N*PRE) − 83.23(N*YR1) + 384.43(N*YR2) + 286.24, **0.16**
N	5	0.99	0.16	0.70	−328.67	$y = -55.88$(N) + 331.92, 0.02
P	5	1.00	0.16	0.86	−328.67	$y = -55.63$(P) + 331.92, 0.02
N : P	5	1.72	0.11	0.97	−329.04	$y = -6.32$(N : P) + 331.92, 0.04

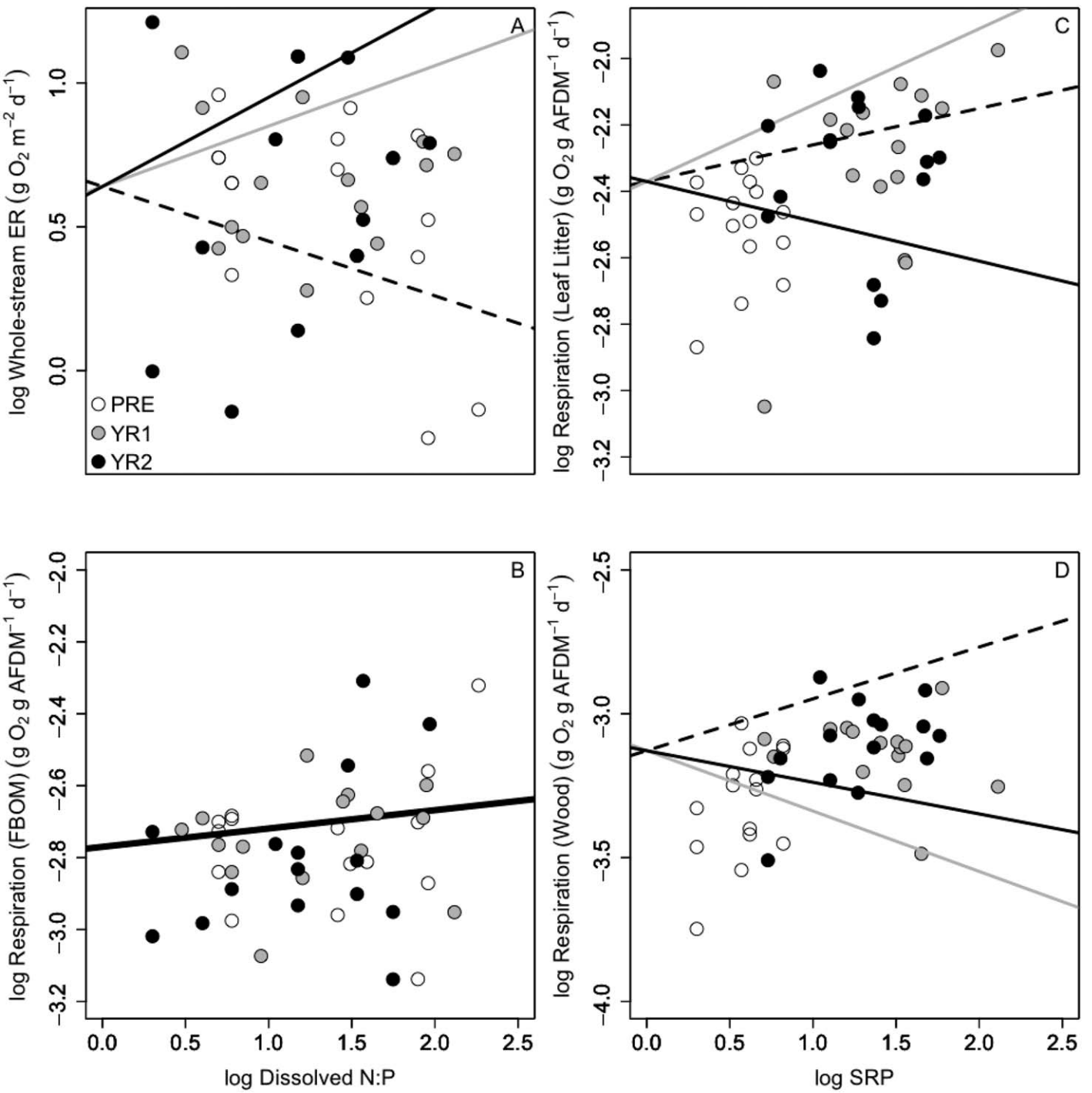

Fig. 3. Log-log correlation plots from top ranking linear mixed-effects models comparing fixed effects of molar N : P ratios on (**A**) whole-stream ER and (**B**) FBOM respiration, and the fixed effects of concentrations of SRP on microbial respiration rates associated with (**C**) leaf litter and (**D**) wood) from $n = 5$ streams during pre-enrichment (PRE) and nutrient enrichment years (YR1, YR2). Effects of stream nested in year were included as random factors in models. Absolute values of ER were calculated before log-transformation. Lines represent slopes fitting response variables. Lines correspond to year (dashed black, PRE; solid gray, YR1; solid black, YR2; thick solid black, all years). Model selection was based on delta $AIC_c \leq 2$ and conditional R^2 (*see* Table 3). Here, we plot the best-fit model for each response variable. Whole-stream ER (**A**) and respiration rates on FBOM (**B**) were most influenced by dissolved N : P. Respiration rates on leaf litter (**C**) and wood (**D**) were most affected by SRP.

Table 4. Whole-stream FBOM, leaf litter, and wood standing stocks in the five experimental streams during PRE and enrichment years (YR1, YR2). Seasonal mean FBOM and leaf litter standing stocks (AFDM; g AFDM m^{-2}) were determined from replicate collections (FBOM $n = 4$ and leaf litter $n = 8$, monthly throughout the year) from benthic transects (leaf litter) and benthic cores (FBOM) that were randomly chosen from along an upstream–downstream gradient in each stream. Wood standing stocks (g AFDM m^{-2}) were measured once in each stream from benthic transects ($n = 15$). Stream-specific values of wood standing stocks were 618 (N : P 2), 537 (N : P 8), 876 (N : P 16), 448 (N : P 32), and 469 (N : P 128).

		FBOM (g AFDM m^{-2})				Leaf litter (g AFDM m^{-2})			
Stream targeted N : P	Year	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
2	PRE	51.6	152.9	143.5	109.4	455.3	504.1	421.5	902.4
	1	94.4	96.8	85.5	99.5	82.6	99.6	389.8	322.0
	2	90.8	70.3	80.1	80.0	55.6	53.0	308.7	229.2
8	PRE	34.4	40.7	74.7	47.2	152.8	150.6	502.4	411.9
	1	60.0	58.1	107.2	45.2	61.5	50.0	288.7	135.5
	2	30.3	28.8	53.0	30.8	45.3	26.7	378.5	212.3
16	PRE	123.1	74.8	137.4	84.8	294.7	325.7	445.9	439.7
	1	60.9	84.7	82.2	125.3	83.5	108.6	335.9	404.9
	2	82.8	67.0	84.5	75.6	52.0	39.4	445.6	138.3
32	PRE	43.0	121.0	39.8	66.9	273.9	293.0	213.9	412.6
	1	60.4	39.3	56.8	89.9	52.7	70.1	251.0	251.1
	2	40.5	52.0	27.0	40.9	31.6	22.7	340.0	210.5
128	PRE	75.0	81.7	62.6	40.1	195.0	249.6	271.9	285.6
	1	43.6	61.6	48.3	37.1	66.7	46.7	253.0	232.4
	2	36.4	37.9	32.7	24.0	35.1	24.9	380.9	228.4

enrichment increased measured concentrations of DIN (range = 50–436 $\mu g\ L^{-1}$) and SRP (range = 5–75 $\mu g\ L^{-1}$), both of which closely matched target concentrations (DIN range = 81–650 $\mu g\ L^{-1}$; SRP range = 11–90 $\mu g\ L^{-1}$) in YR1 and YR2 of the experiment (Table 1). Mean temperature varied by up to 2.5°C across streams and years (Table 1). Mean discharge ranged from 1.5 L s^{-1} to 20.0 L s^{-1}, depending on stream and year (Table 1). Mean light integration ranged from 0.2 mol $m^{-2}\ d^{-1}$ to 3.2 mol $m^{-2}\ d^{-1}$, depending on stream and year (Table 1).

Whole-stream ecosystem metabolism

Added N and P generally increased rates of ER (range = −0.6 g $O_2\ m^{-2}\ d^{-1}$ to −16.3 g $O_2\ m^{-2}\ d^{-1}$) above PRE levels in YR1 and YR2, particularly in summer (Table 2). Before (PRE) and during YR1 and YR2 of enrichment GPP was low (range = 0.0–0.4 g $O_2\ m^{-2}\ d^{-1}$; Table 2). The amount of wood per m^2 was a significant predictor of ER and the relationship between wood standing stock and ER became steeper with nutrient enrichment. Wood standing stocks in PRE (Adj. $R^2 = 0.20$, $p = 0.05$), and YR2 (Adj. $R^2 = 0.41$, $p < 0.01$) were correlated with higher whole-stream ER (Fig. 2). Whole-stream ER was not explained by other detrital POM standing stocks (FBOM and leaf litter) in PRE, YR1, or YR2 (all Adj. $R^2 < 0.01$, $p > 0.05$). Whole-stream ER normalized by wood standing stocks was similar among streams during nutrient enrichment ($p = 0.55$).

The best-supported models of ER included dissolved N : P alone and interacting with year, or P alone, explaining 20–24% of variance in ER (Table 3; Fig. 3A). Prior to nutrient enrichment, ER was higher at lower N : P, but during enrichment ER increased with increasing N : P (Fig. 3A). The best-supported model of whole-stream GPP (explaining ~ 14% of variance) included year alone (Table 3).

Substrate-specific microbial respiration rates and fungal biomass

Substrate-specific respiration (R) rates associated with FBOM (range: 0.001–0.005 g O_2 g $AFDM^{-1}\ d^{-1}$) were variable before and during enrichment (Fig. 3B), whereas R associated with leaf litter (range: 0.001–0.01 g O_2 g $AFDM^{-1}\ d^{-1}$) and wood (range: 0.0002–0.001 g O_2 g $AFDM^{-1}\ d^{-1}$) increased with nutrient enrichment (Fig. 3C,D). Specifically, R on leaf litter increased in YR1 and YR2 compared to PRE, and leaf litter R was higher with increasing P in PRE and YR1 but decreased in YR2 with increasing P (Fig. 3C; Table 3). Respiration rates on wood increased in YR1 and YR2 compared to PRE, but rates declined with increasing P in YR1 and YR2 (Fig. 3D; Table 3). Dissolved N : P explained the most variance in R associated with FBOM (15%), whereas interactions between dissolved P and year best explained differences in R associated with leaf litter (25%) and wood (35%; Table 3). Fungal biomass associated with leaf litter (range: 6.1–

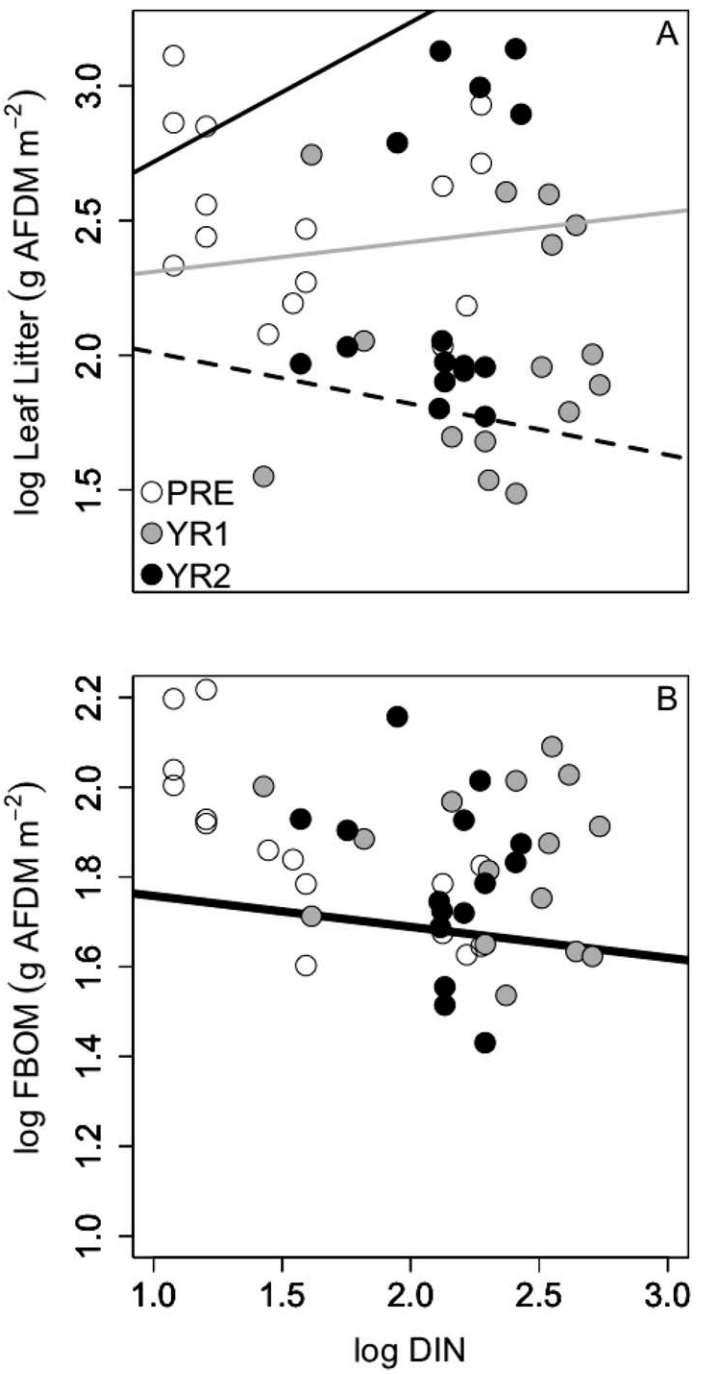

Fig. 4. Log-log correlation plots from top ranking linear mixed-effects models comparing fixed effects of concentrations of DIN on standing stocks of (**A**) leaf litter and (**B**) FBOM from $n = 5$ streams during pre-enrichment (PRE) and nutrient enrichment years (YR1, YR2). Effects of stream nested in year were included as random factors in models. Lines represented as in Fig. 3. Model selection was based on delta $AIC_c \leq 2$ and conditional R^2 (*see* Table 3). Here, we plot the best-fit model for each response variable.

69.7 mg g AFDM^{-1}) and wood (range: 4.7–27.6 mg g AFDM^{-1}) did not change with added N and P (leaf litter: $F_{2,42} = 1.43$, $p = 0.25$; wood: $F_{2,41} = 0.65$, $p = 0.53$).

Scaled substrate-specific R (g O_2 g m^{-2} d^{-1}) did not explain whole-stream ER in PRE, YR1, or YR2 (all Adj. $R^2 < 0.01$, $p > 0.05$). Area-specific fungal biomass scaled to whole streams based on leaf litter and wood standing stocks (g m^{-2}) did not increase with added N and P ($F_{2,40} = 0.98$, $p = 0.38$) and did not explain whole-stream ER in PRE, YR1, or YR2 (all Adj. $R^2 < 0.01$, $p > 0.05$).

Detrital POM standing stocks

Total detrital POM standing stocks (FBOM, leaf litter, and wood) ranged from 520 g AFDM m^{-2} to 1630 g AFDM m^{-2} among the five streams (Table 4). Wood standing stocks (one-time estimate) varied among streams by up to 2× (Table 4). Nutrient enrichment generally reduced FBOM and leaf litter standing stocks and their differences among streams (Table 4). We detected interactive effects of added N and year on leaf litter standing stocks, in which leaf litter generally declined with increasing N concentrations despite high autumn inputs in YR1 and YR2 (Fig. 4A; Table 3). Added N and dissolved N : P explained 31–35% of variance in FBOM standing stocks, and FBOM generally declined with increasing N concentrations across all years (Fig. 4B; Table 3).

Temporal variation in responses

Prior to nutrient enrichment, intra-annual variation in standing stocks of FBOM and leaf litter was similar (Fig. 5). Added N and P increased within-year variation of leaf litter standing stocks ($F_{2,12} = 20.8$, $p < 0.01$) compared to PRE (Fig. 5); within-year variation of FBOM ($F_{2,12} = 0.2$, $p = 0.8$) and fungal biomass ($F_{2,12} = 2.0$, $p = 0.2$) was unaffected by enrichment (Fig. 5). Intra-annual variation in scaled substrate-specific R increased during nutrient enrichment ($F_{2,12} = 6.8$, $p = 0.01$), whereas differences among streams declined. In contrast, whole-stream ER became strongly more variable among streams with added N and P, outweighing any differences in intra-annual variation among streams ($F_{2,12} = 0.01$, $p = 0.99$; Fig. 5).

Discussion

We predicted that added N and P would increase heterotrophy during periods of high detritus availability (i.e., after leaf-fall in autumn), driven by stimulation of heterotrophic processing of detrital POM (Fig. 1), as well as increase autotrophy during periods of higher temperature and light availability (i.e., before leaf-out in spring). Nutrients generally increased whole-stream ER, but GPP was very low throughout the study and was unaffected by nutrient enrichment. We also predicted that microbial to ecosystem-scales of metabolic processes would be co-limited by N and P. Although detrital POM standing stocks declined and associated respiration rates increased with nutrient enrichment, as expected, responses were driven more by N than P. Prior to nutrient enrichment, ER was higher at lower N : P, but during

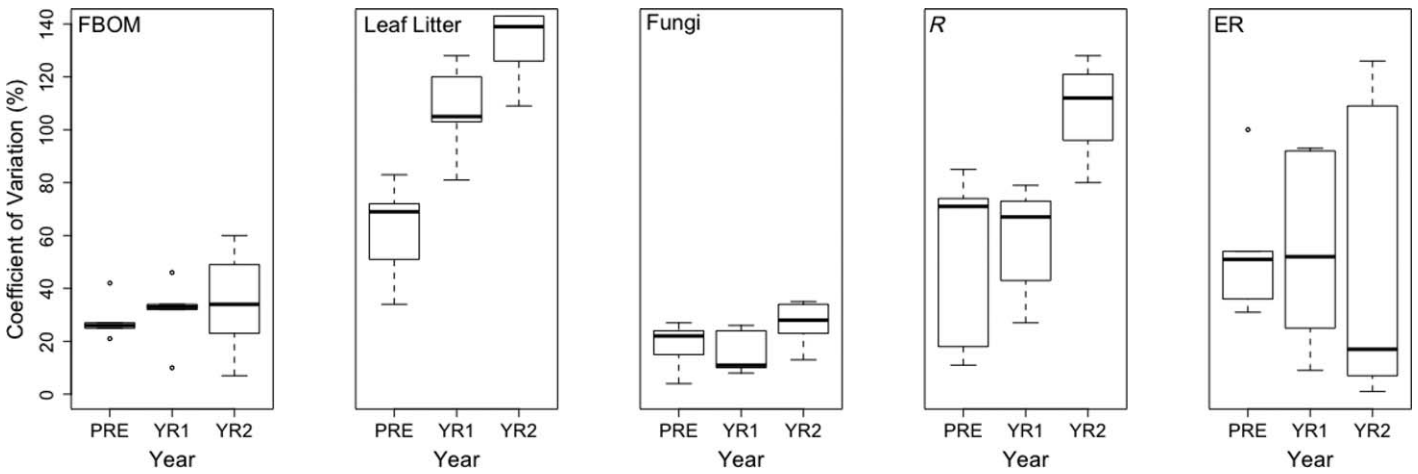

Fig. 5. Coefficients of variation for FBOM and leaf litter standing stocks (g AFDM m^{-2}), areal-specific fungal biomass (g m^{-2}), and scaled substrate-specific (*R*) and whole-stream ER (g O_2 m^{-2} d^{-1}) across seasons (except winter) and streams ($n = 5$). Intra-annual variation in FBOM and leaf litter was estimated from seasonal averages (except winter) of monthly samples. Fungal biomass (fungi) was measured from seasonal (except winter) samples of decomposing leaf litter and wood. Fungi and *R* scaled to whole streams were based on standing stocks of FBOM (except for fungal biomass), leaf litter, and wood (one-time estimate). Whole-stream ER values were seasonal (except winter) estimates from single-station, diel measures of DO from each of the five streams.

enrichment ER increased with increasing N : P. Increased heterotrophy from microbial to ecosystem scales occurred at concentrations of N and P that are now common among pristine and human-impacted ecosystems (Alexander and Smith 2006). Across these low-to-moderate concentrations, N and P differentially explained substrate-specific and ecosystem-level heterotrophic responses, but a consistent N : P ratio effect was not measured. Biological responses to nutrient enrichment vary across spatial and temporal scales (Rosemond et al. 2015), which likely explain how heterotrophic processes that affect stream C loss differentially respond to changes in N and P.

Variation in whole-stream ER increased with added N and P. Streams with the highest magnitude and variation in ER also had the highest detrital POM standing stocks, specifically of wood. Wood standing stocks varied by up to 2× among streams, likely contributing to higher and more variable whole-stream ER. Retention of slow-turnover, recalcitrant forms of POM, such as wood, can increase variability in stream organic matter processing by promoting accumulation of both labile and recalcitrant forms of POM (Bilby and Likens 1980). When accounting for how added N and P interact with detrital POM and scaled substrate-specific microbial respiration among streams, we observed higher seasonal variation in scaled substrate-specific respiration and higher among-stream variation in whole-stream ER. Larger increases in respiration and detrital processing rates with added nutrients have been associated with wood and recalcitrant leaf litter (Gulis et al. 2004; Greenwood et al. 2007), indicating that stored POM or POM with long residence times contributes to enhanced heterogeneity in stream metabolic demand and that this variation increases with nutrient availability. Experimental additions of wood tend to increase organic matter accumulation and burial in streams (Entrekin et al. 2008; Flores et al. 2011), which can increase structural and functional heterogeneity. Although nutrients reduced seasonal variation in whole-stream ER, specifically by increasing ER in spring and summer above pre-enrichment levels, increased variation in ER among streams during nutrient enrichment is likely explained by differences in stored vs. entrained detrital POM driven by wood availability.

We observed similar seasonal responses to nutrient enrichment among different detrital POM types. We quantified nutrient-induced POM loss from streams through enhanced ER and detrital processing. Nutrient-induced leaf litter loss was more pronounced than loss of FBOM. Seasonal standing stocks of detrital POM generally declined with experimental enrichment, but effects of N and P differed compared to previous assessments of annual leaf litter loss rates (Rosemond et al. 2015). Experimental N and P additions accelerated whole-stream leaf litter loss, and reductions in seasonal leaf litter standing stocks were explained more by N than by P. Previous assessment of the same five streams found similar effects of both added N and P on leaf litter loss rates, measured as changes in monthly standing stocks across an annual cycle from maximum to minimum quantity (Rosemond et al. 2015). Our finding of stronger N than P effects on seasonal leaf litter standing stocks may be due to our exclusion of data from winter months when we could not measure whole-stream ER to relate to detrital POM. However, declines in FBOM standing stocks were also consistently driven more by N than P. Fungal biomass in detritus-based streams is higher on leaf litter than on FBOM or wood (Gulis et al. 2008; Tant et al. 2013), and N may be more

important than P for fungal biomass accrual on litter (V. Gulis unpubl.). However, fungal colonization of leaf litter interacts with added P to decrease litter C:P and increase invertebrate-mediated litter breakdown rates (Manning et al. 2015). Empirical tests in laboratory microcosms indicate that litter C:P and fungal biomass may be decoupled, as fungi may store P, driving declines in leaf litter C:P, while utilizing N for growth (V. Gulis unpubl.). Overall, we found that N was a better predictor than P of seasonal (excluding winter) detrital standing stocks.

We expected whole-stream ER to be explained by substrate-specific R and fungal biomass scaled to whole streams based on POM standing stocks. Scaled substrate-specific microbial respiration of detrital POM (FBOM, leaf litter, and wood) was insufficient to explain whole-stream ER, suggesting that we were unable to estimate detrital POM storage and associated microbial activity comprehensively in our streams. Microbial respiration associated with FBOM that is dynamically transported from headwater streams, as well as respiration on buried detrital POM, was not captured by our seasonal substrate-specific respiration measurements, yet represent potentially important contributors to whole-stream ER (Mulholland et al. 2001). Buried detrital POM and FBOM are predominantly colonized by bacteria (Tant et al. 2013), and it is possible that bacterial activity on these detrital resources was enhanced by added N and P. The lack of a clear pattern between whole-stream ER and areal-specific fungal biomass with added N and P is likely explained by rapid loss of leaf litter standing stocks during enrichment. Leaf litter breakdown rates in these study streams are driven by interactions between dissolved nutrient concentrations and fungi colonizing litter (Manning et al. 2015; Manning et al. 2016). Mass-specific respiration rates associated with leaf litter were an order of magnitude higher than those for FBOM and wood, and rapid declines in leaf litter standing stocks and associated fungal biomass with added N and P equate to large declines in contributions of microbial R associated with leaf litter to whole-stream ER.

Capturing variation in stream ecosystem metabolism requires measurements at multiple spatial and temporal scales. The use of periodic measurements of stream metabolism at baseflow conditions has been effective at characterizing general differences among streams in different biomes and across land-use types (Mulholland et al. 2001; Bernot et al. 2010). Continuous measurements of daily metabolism are now common because of increased use of optical DO sensor technology, and enhanced temporal resolution enables measurements of whole-stream metabolism throughout periods from low-to-high-flow conditions (Roberts et al. 2007). We compared integrated diel metabolism to seasonal averages in pre-treatment and enrichment years, showing that nutrient enrichment increased mean and variance of whole-stream ER. However, our inability to capture whole-stream ER during the winter in these cold, turbulent streams limits our understanding of how added N and P accelerated stream C loss during that season. In addition, the potential for groundwater to bias estimates of ER can be high if groundwater oxygen concentrations are low (McCutchan et al. 1998; Hall and Tank 2005). Although we did not explicitly measure groundwater oxygen concentrations, our near-saturated surface-water oxygen concentrations indicate that either groundwater oxygen is high or not substantially reducing surface water oxygen. However, whole-stream metabolism measurements that incorporated winter data and groundwater inputs would have improved our estimates of C losses from ER in these ecosystems.

The results of our large-scale, multi-stream nutrient addition experiment expand theoretical predictions of elemental limitation among ecosystems (Elser et al. 2007). Unlike ecosystems dominated by primary producers, donor-controlled ecosystems like detritus-based headwater streams are dominated by heterotrophic consumers, where added nutrients have been repeatedly shown to accelerate C loss through enhanced microbial respiration and macroinvertebrate feeding associated with leaf litter (Benstead et al. 2009; Suberkropp et al. 2010; Rosemond et al. 2015). Although forest streams store large amounts of C, increases in nutrient availability rapidly reduce surficial POM stocks (Benstead et al. 2009; Rosemond et al. 2015), decreasing the long-term C storage capacity as well as the basal energy source for food web production in these donor-controlled ecosystems. Our findings emphasize the importance of ecosystem C retention in maintaining ecosystem function and the potential for long-term vulnerability to sustained C losses in the face of near ubiquitous elevated N and P concentrations in surface waters (Alexander and Smith 2006).

Streams and rivers are important sources of CO_2 to the atmosphere and are critical components of the global C cycle (Cole et al. 2007; Battin et al. 2008; Raymond et al. 2013). We show that nutrient enrichment, which is widespread, can increase processing of detrital POM and CO_2 flux from stream ecosystems. Our study suggests that in-stream processes stimulated by nutrients could increase C fluxes, with relatively unknown consequences for downstream ecosystems and long-term C retention (Hotchkiss et al. 2015). The current estimate of global CO_2 evasion from inland waters is 2.1 Pg C y^{-1}, with uncertainty around this estimate currently driven by the paucity of measurements (Raymond et al. 2013). Streams and rivers are known hotspots for POM processing and CO_2 flux (Borges et al. 2015; Hotchkiss et al. 2015; Rosemond et al. 2015; Demars et al. 2016), but we lack high spatial resolution of stream and river contributions to river networks, as well as temporal resolution of biogeochemical processes in streams and rivers (Benstead and Leigh 2012; but see Hall et al. 2015). Expanding our measurements of dynamic processes in small streams and rivers across gradients in nutrients and temperature (Demars et al. 2016; Williamson et al. 2016) will enhance our understanding of

the role of river networks in global elemental cycles (Benstead and Leigh 2012).

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Conflict of Interest

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