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PRIMARY RESEARCH PAPER

Acidification, stress, and detrital processing: implications for ecosystem function in headwater streams

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Abstract Environmental influences like acidification promote stress at the ecosystem level that manifests as reduction in metabolic and biogeochemical efficiency. Headwater streams along a chronic acidity gradient were assessed to explore how stress alters microbial abundance and activity and their influence on ecosystem structure and function. Streams draining deciduous forests were investigated during autumn when channels were filled by leaf litter. Whole-system measures of respiration were coupled to estimates of fungal biomass in leaf biofilms to generate an ecosystem-level measure of metabolic efficiency (qCO_{2E}, g CO_2–C g C^{-1} d^{-1}). Stable isotope releases of nitrate nitrogen (^{15}N–NO_3) were performed to address nitrate uptake (U_{NO_3}) across streams. Fungal stocks decreased across five streams as pH declined (6.98–5.34). Whole-system respiration decreased fivefold with increasing acidity, while qCO_{2E} did not respond consistently to acidification, but was correlated with stream temperature. Across streams, concentrations of nitrogen (N) were low and U_{NO_3} related to nutrient availability and not to stream acidity. Results illustrate that acidification alters ecosystem processes through influences on microbial abundance and metabolic activity, while scarce N availability and low U_{NO_3} characterized biogeochemical behavior during autumnal periods of maximal detrital stocks.

Keywords Metabolic efficiency · N uptake · Stress · Acidification · Streams · Fungi

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Introduction

Acidification of surface waters is a historical and ongoing aspect of global change with the most severe forms of stream acidification (i.e., pH < 3) associated with acid mine drainage (Hogsden & Harding, 2011). Atmospheric deposition typically results in less severe stream acidification (Herlihy et al., 1993), but episodic acidification from soil waters can drive stream pH below 4 (Kahl et al., 1992). Following reduced industrial emissions (Lynch et al., 2000; Fowler et al., 2005; Greaver et al., 2012), many streams

worldwide have chemically rebounded to significant degrees (Evans et al., 2001; Skjelkvåle et al., 2005; Helliwell et al., 2014; Kopáček et al., 2015), but ecological recovery has been less widespread (Wright et al., 2005).

In running waters, a range of ecological responses are generated by acidification including altered food web characteristics (Layer et al., 2010, 2013), degradation of fish populations (Mulholland et al., 1992), and overall reductions in lotic biodiversity (Lovett et al., 2009). At the ecosystem level, research has shown that reduced rates of organic matter processing (i.e., litter decomposition) in acidified streams (Mulholland et al., 1987; Dangles & Guérold, 1998; Simon et al., 2009) are tied to loss of invertebrate decomposers (Dangles et al., 2004a; Layer et al., 2013) and microbial impairment, including decreased fungal diversity (Baudoin et al., 2008), abundance, and respiration rates (Dangles et al., 2004a). Understanding acidification influences on litter processing is of particular interest since autumnal leaf fall represents an important subsidy of terrestrial organic matter to streams of forested landscapes. Leaf litter inputs drive ecosystem functioning by promoting periods of intense respiratory activity (Roberts et al., 2007) and nutrient consumption that can sequester all available nitrogen (N) and phosphorus (P) in nutrient-poor systems (Mulholland et al., 1985; Valett et al., 2008).

Gradual but directional alterations to environmental conditions, like those associated with acidification, are stressful to biota because they challenge organismal homeostasis and impose physiological costs (Schimel et al., 2007). Stress at the ecosystem level has been defined as sustained reduction in metabolic and biogeochemical efficiency (Odum, 1985; Schimel et al., 2007). Wardle & Ghani (1995) provided the metabolic quotient (qCO_2, biomass-specific microbial respiration) as an indicator of stress in soil systems where increasing qCO_2 reflected greater allocation of carbon (C) to respiration per unit biomass and decreased growth efficiency. In their study, qCO_2 increased (i.e., efficiency declined) with increasing soil acidity.

Beyond acidity alone, the potential exists for additive or synergistic interactions among stressors (Crain et al., 2008; Townsend et al., 2008), emphasizing the need to address simultaneous factors of natural or anthropogenic origin. Ecosystem recovery from environmental stressors like acidification will likely reflect energetic and resource constraints imposed by thermal regimes and resource supplies characteristic of the broader landscapes within which the systems reside. For example, Jenkins et al. (2013) showed that temperature and pH interacted to influence decomposition rates in streams after 30 years of recovery from acidification.

With these issues in mind, we conducted an ecosystem-level assessment of how chronic acidification influences metabolism and uptake of dissolved N during times of maximal leaf stocks within streams spanning a pH gradient representative of acid conditions typical of those resulting from atmospheric deposition. We focused on ecosystem-level manifestation of stress by addressing energy flow and N cycling through microbial biofilms associated with decaying leaves, a substrate–microbe complex central to ecosystem functioning in running waters (Gessner & Chauvet, 1993). We performed short-term ^{15}N releases of nitrate (NO_3^-) to quantify N uptake (U_{NO_3}) and measured ecosystem respiration (ER) in five streams across which chronic pH values ranged from ca. 7 to 5.3 in the Appalachian Mountains, USA. By coupling ER to standing stocks of leaf litter and fungal biomass of leaf biofilms, we generated an ecosystem metabolic quotient (qCO_{2E}) to reflect rates of energy flow normalized to abundance of the dominant decomposers.

Based on the anticipated influences of resource, metabolic, and stress constraints, we addressed the following predictions: (1) whole-stream respiration (i.e., ER) should decline with increasing acidity, (2) ecosystem metabolic quotient (qCO_{2E}) should increase with temperature following Arrhenius relationships as predicted by metabolic theory (Brown et al., 2004; Cross et al., 2015), (3) qCO_{2E} will increase with acidity (i.e., declining pH, base cation concentration, or acid neutralizing capacity, ANC) reflecting decreased metabolic efficiency, and (4) areal uptake of N should decrease with increasing acidity reflecting microbial impairment (Ferreira & Guerold, 2017) in acidified streams.

Materials and methods

Study sites

The study was conducted in 5 second- to fourth-order streams draining catchments within Shenandoah National Park (SNP), Virginia, USA (Online Resource 1) which comprises ~ 80,000 ha of predominantly (95%) forested land and has been an active location for acid deposition research since 1979 (http://swas.evsc.virginia.edu). Enhanced acid deposition is due to the park's position downwind of the Ohio River Valley's coal-fired power plants (Driscoll et al., 2001). Differences in stream pH arise within the park from variation in underlying geology and subsequent capacity for acid neutralization (Online Resource 1). Five study streams, distributed across 3 major bedrock types, represent an acidity gradient with pH ranging from 6.98 to 5.34 based on long-term mean values derived from quarterly samplings, 1993–2008 (Table 1, James N. Galloway, University of Virginia, unpublished data). At the time of study (November and December 2007), measured pH differed from long-term means by less than one 0.1 pH units, streams were shallow (depth 10–25 cm), relatively small (width 4.9–7 m), and flow ranged from 15.4 to 40.5 l s^{-1} (Table 1). Stream channels were inundated with leaf litter representing a mix of species but predominantly Chestnut Oak (*Quercus prinus*). Despite acidification driven by atmospheric sources and historically elevated N deposition, nutrient concentrations were low (Table 1) with both dissolved inorganic N (predominantly NO_3–N) and soluble reactive P (SRP) less than 5 μg l^{-1} for all samples, typical of unenriched forested streams during and immediately following autumn leaf fall (e.g., October–December, Mulholland et al., 1985).

Table 1 Flow characteristics and physicochemistry of the five study streams

Parameter	Meadow Run	Paine Run	Brokenback Run	Hazel River	Piney River
Flow conditions					
Wetted width (m)	5.2	4.9	5.9	4.2	7
Depth (cm)	10.0	25.0	16.0	18	12.0
Velocity (cm s^{-1})	2.9	1.3	1.8	5.2	4.2
Discharge (l s^{-1})	15.4	16.1	33.0	40.5	34.3
Physicochemistry					
pH	$5.34^{a} \pm 0.05$ (16)	$5.71^{b} \pm 0.02$ (54)	$6.45^{c} \pm 0.06$ (15)	$6.55^{d} \pm 0.05$ (15)	$6.98^{e} \pm 0.02$ (51)
Temperature (°C)	$5.22^{b} \pm 0.11$ (24)	$6.84^{a} \pm 0.17$ (24)	$5.24^{b} \pm 0.07$ (24)	$4.73^{c} \pm 0.09$ (24)	$3.13^{d} \pm 0.06$ (24)
Cations					
Ca^{2+} (mg l^{-1})	$0.81^{a} \pm 0.10$ (12)	$0.98^{a} \pm 0.53$ (12)	$2.23^{bc} \pm 0.05$ (12)	$2.13^{b} \pm 0.05$ (12)	$4.70^{c} \pm 0.15$ (11)
Mg^{2+} (mg l^{-1})	$0.36^{a} \pm 0.05$ (12)	$0.54^{bc} \pm 0.03$ (12)	$0.43^{ab} \pm 0.01$ (12)	$0.48^{ab} \pm 0.02$ (12)	$1.31^{c} \pm 0.04$ (11)
K^{+} (mg l^{-1})	$0.89^{bc} \pm 0.05$ (12)	$1.69^{c} \pm 0.08$ (12)	$0.39^{a} \pm 0.02$ (12)	$0.43^{ab} \pm 0.02$ (12)	$0.26^{a} \pm 0.03$ (11)
Na^{+} (mg l^{-1})	$0.58^{a} \pm 0.04$ (12)	$0.66^{a} \pm 0.05$ (12)	$1.60^{b} \pm 0.03$ (12)	$1.68^{b} \pm 0.06$ (12)	$2.09^{c} \pm 0.10$ (10)
Base cations (μeq l^{-1})	$109^{a} \pm 5$ (12)	$157^{b} \pm 6$ (12)	$226^{c} \pm 4$ (12)	$230^{c} \pm 6$ (12)	$438^{d} \pm 16$ (10)
ANC (μeq l^{-1})	$4.5^{a} \pm 0.4$ (16)	$13.0^{b} \pm 0.5$ (54)	$96.3^{c} \pm 2.7$ (15)	$105.1^{d} \pm 2.6$ (15)	$242.0^{e} \pm 7.8$ (51)
Nutrients					
NH_4-N (μg l^{-1})	bdl	bdl	bdl	bdl	bdl
NO_3-N (μg l^{-1})	$1.1^{a} \pm 0.2$ (11)	$0.9^{a} \pm 0.7$ (11)	$2.4^{b} \pm 0.7$ (8)	$2.0^{b} \pm 1.0$ (8)	$1.1^{a} \pm 0.8$ (12)
SRP (μg l^{-1})	$0.7^{a} \pm 0.1$ (12)	$1.5^{a} \pm 0.2$ (12)	$4.8^{c} \pm 0.5$ (11)	$4.5^{c} \pm 0.2$ (12)	$2.9^{b} \pm 0.1$ (12)
DOC (mg l^{-1})	$0.76^{ab} \pm 0.08$ (11)	$0.70^{a} \pm 0.07$ (9)	$2.12^{c} \pm 0.27$ (11)	$2.14^{c} \pm 0.11$ (12)	$1.28^{bc} \pm 0.5$ (7)

Data are mean ± standard error (*n*) except for pH (long-term average values) and temperature (mean value from automated sonde). Values in the same row with distinct superscripts are significantly different (Tukey's HSD, $P < 0.05$)

bdl below detection limit (< 1 μg l^{-1})

Solute release experiments

We conducted a single continuous addition (3–5-h duration) of 99% ^{15}N-enriched KNO_3, along with chloride (Cl) as a conservative tracer, to each stream between November 27 and December 14, 2007, times of maximal leaf litter standing stock in Appalachian streams (Suberkropp et al., 2010). Prior to releases, we collected background water samples ($n = 3$) at each of four transects along each reach (60–100 m) for analysis of NO_3–N, ammonium–N (NH_4–N), SRP, dissolved organic carbon (DOC), Cl, base cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), and ^{15}N–NO_3. Releases of $K^{15}NO_3$ were designed to increase the delta ^{15}N of stream water by 500‰ at the addition site.

Once steady-state conditions were achieved (as indicated by constant values for specific electrical conductivity, hereafter conductivity, μS cm^{-1}) at the most downstream transect, three replicate water samples were collected at each transect, filtered (Whatman GF/F, pore size = 0.7 μm) into acid-washed 125-ml polyethylene bottles, and frozen until analyzed for Cl and NO_3–N. Additional samples for analysis of ^{15}N–NO_3 were collected in acid-washed 1-l bottles and kept on ice until filtered in the laboratory, and refrigerated ($\sim$ 4°C) until processed.

Temperature (°C), conductivity, and dissolved oxygen (DO) of stream water were continuously monitored at 5-min intervals using an automated sonde (Hydrolab Model 4a, Hydrolab, Hach Environmental, Loveland, Colorado, USA) at the most downstream transect. Stream temperature and conductivity were represented as the mean of hourly values derived from the 5-min measures (i.e., $n = 24$/stream). Velocity and stream discharge were determined from the conservative tracer curves and dilution gauging techniques for each transect (Webster & Valett, 2007). Wetted widths and water depths were measured at 10-m intervals along each study reach.

Water sample processing and analysis

We followed the reduction–diffusion method (Sigman et al., 1997; Mulholland et al., 2008) to process ^{15}N–NO_3 samples. Briefly, following removal of ammonium via volatilization, NO_3^- was reduced to ammonium by Devarda's Alloy, liberated in a basic solution (MgO), and captured on an acidified filter. Filters were encapsulated and analyzed for ^{15}N:^{14}N ratios on a Europa Integra mass spectrometer (Sercon, Cheshire, UK) at the University of California, Davis Stable Isotope Facility.

Concentrations of NO_3–N, NH_4–N, and SRP were determined following standard methods (APHA, 2005, detection limit equals 1 μg l^{-1} as N or P) via the diazo, modified phenate, and molybdate-antimony methods, respectively, using a Lachat QuikChem 8500 auto analyzer (Lachat Instruments, Loveland, Colorado, USA). DOC was quantified with persulfate digestion on an OI Analytical 1010 Total Organic Carbon Analyzer (Oceanographic International, College Station, Texas, USA). Chloride and base cations were measured using a Dionex DX 500 Ion Chromatograph (Dionex, Sunnyvale, California, USA). Long-term pH measures and acid neutralizing capacity (ANC, measured on unfiltered samples using Gran titrations) for each stream was represented by means derived from quarterly (autumn) samples taken from each stream (Table 1, $n = 15$–54) over the 1993–2008 period of collection (J. Galloway, University of Virginia, unpublished data). These long-term pH values were nearly identical to measurements taken during N uptake and respiration assays. While aluminum (Al) is well known to be influential in acidified ecosystems, it was not addressed here since our earlier work in these streams indicated that concentrations are low during baseflow and that experimental acute additions of Al had no detectable influence on leaf biofilm respiration or N uptake (Ely et al., 2010).

Leaf litter and fungal biomass

Ten samples (0.25 m^2) of leaf litter were collected at random locations along each study reach using open-bottom cylinders. Leaf material and associated organic matter (OM) was removed by hand, sieved (8 mm), and larger particles (excluding wood) retained until processing. Samples were dried at 55°C (24 h), weighed, ashed (4 h, 550°C), and reweighed to obtain ash-free dry mass (AFDM) as a measure of leaf litter standing stock (g AFDM m^{-2}).

A subset ($n = 5$) of the litter samples in each stream was chosen for analysis of ergosterol to estimate fungal biomass. For each sample, we cut $\sim$ 50 leaf discs from randomly chosen, recognizable leaf material. Five of these leaf discs (40–60 mg AFDM) were then kept in 5 mL methanol (HPLC grade) and frozen at − 20°C until measurement of ergosterol content.

Ergosterol was extracted in methanol and quantified using high-pressure liquid chromatography (Tank et al., 1998). Ergosterol mass was multiplied by a conversion factor of 182 (Gessner & Chauvet, 1993) to estimate fungal biomass (FB; mg biomass g^{-1} AFDM), recognizing the potential for this ratio to vary with growth condition among streams (Charcosset & Chauvet, 2001).

Ecosystem respiration and qCO_{2E}

Concurrent with ^{15}N releases, ER was measured using open-system single-station analyses of diel DO curves (Marzolf et al., 1994) derived from the automated sondes placed at the bottom of each reach. Equilibrated and calibrated sondes were retrieved after 24 h and placed back into water-saturated air to correct for drift. Data revealed no evidence of primary production during daytime hours, consistent with the lack of observable stream bed and lack of algae on leaf surfaces. ER for each 5-min interval during the night was calculated as the change in DO corrected for atmospheric exchange determined as the product of the reaeration coefficient and the oxygen deficit. Reaeration coefficient was determined using sulfur hexafluoride as a volatile tracer (Marzolf et al., 1994). Daytime ER was extrapolated from average ER during 1-h pre-dawn and 1-h post-dusk periods. Values for ER were summed over 24 h to yield daily rates (g O_2 m^{-2} d^{-1}).

For each stream, FB (mg g^{-1} AFDM) was multiplied by average litter standing stock (g AFDM m^{-2}) to quantify fungal standing stock (FSS, g OM m^{-2}). Fungal C (FC, g C m^{-2}) was derived assuming that C comprised 50% of OM mass. Ecosystem metabolic quotient (qCO_{2E}, g CO_2–C g^{-1} fungal C d^{-1}) was determined by multiplying ER by a respiratory quotient of 0.85 (Bott et al., 2006) and dividing by FC.

During autumnal leaf fall, fungi comprise the vast majority of microbial biomass in streams (Findlay et al., 2002) and dominate respiratory activity (Bergfur & Friberg, 2012). Overview of microbial standing stocks on leaf material indicates that fungal contribution to biomass (Findlay et al., 2002) and C loss is typically an order of magnitude greater than that associated with bacterial biomass (Pascoal & Cássio, 2004). We intentionally targeted autumnal periods when stream channels were filled completely with leaf litter, maximizing fungal standing stocks.

Nitrogen uptake

Nitrate uptake (U_{NO_3}, mg N m^{-2} d^{-1}) was derived using a nutrient spiraling approach (Webster & Valett, 2007). With this method, the uptake length for NO_3^- (S_w, m) is derived following Eq (1):

$$S_w = -\frac{1}{k_L}, \tag{1}$$

where k_L (longitudinal uptake rate, m^{-1}) is the inverse of the slope of the line relating ln (^{15}N flux) and distance downstream (m), associated with the longitudinal decline in water column ^{15}N. Thus, S_w represents the distance traveled by released N before removal from solution. The N mass-transfer coefficient, or uptake velocity (v_f, m/d), normalizes S_w to existing hydrologic conditions, Eq. (2):

$$v_f = \left(\frac{uz}{S_w}\right), \tag{2}$$

where u is measured stream velocity (m d^{-1}) and z is mean depth (m). Combining v_f and ambient NO_3–N concentration (C, mg/m^3) yields areal uptake of NO_3^- (U_{NO_3}, mg N m^{-2} d^{-1}) with Eq. (3):

$$U_{NO_3} = v_f * C. \tag{3}$$

Temperature and Arrhenius metabolic scaling

Temperature data obtained from automated sondes were averaged over the 24-h period corresponding to metabolic measures and used to assess thermal influences on ER. Scaling relationships between ER or qCO_{2E} and stream temperature were addressed using an Arrhenius plot following Enquist et al. (2003) via Eq. (4):

$$\ln(R) = \frac{-E}{1,000\,k}\left(\frac{1,000}{T}\right) + \ln[(b_o)(C)], \tag{4}$$

where R = ER or qCO_{2E}, E is activation energy (0.6 eV), k is Boltzmann constant (8.62×10^{-5} eV K^{-1}), T is stream temperature (K), and b_o and C are normalization constants. Accordingly, Eq. (4) should yield a slope of − 0.6 eV when ln(R) is plotted against $1/kT$ (Enquist et al., 2003), a value shown to be robustly applicable across multiple aquatic systems (Yvon-Durocher et al., 2010). How well metabolic

theory predicts stream metabolism across streams was assessed using the derived slope following linear regression.

Statistical analyses

Differences in pH, temperature, base cations, ANC, nutrients, leaf litter standing stocks, percent OM, and fungal biomass among streams were investigated using one-way analysis of variance (ANOVA). Because each pH value assessed was represented by a single stream, results from ANOVA assessment reflect statistical differences among locations only and cannot be attributed directly to differences in acidity. Tukey's honest significant difference (HSD) was used as a post hoc multiple comparison test following significant ANOVA. Pearson product–moment correlations (r) were used to address relationships among structural variables. We also used least-squares regression models to address continuous responses to increasing stream acidity and among structural and functional measures.

To enable comparison of our results with multiple studies from different locations, we calculated z-scores for fungal biomass following (Eq. 5)

$$z\text{-score} = \frac{(y_i - \bar{y})}{\mathrm{SD}}, \quad (5)$$

where y_i, $\bar{y}$, and SD are, respectively, individual observations, mean values, and standard deviations from each study. This score yields a mean of zero and a SD of one for each study to provide standardized comparative assessment among studies.

Significance level was established at $\alpha = 0.05$ for all statistical tests. For nutrient data, concentrations below detection limit were given values equal to one half of the lowest standard. When necessary, data were ln-transformed to achieve normality and equivalent variances as required for parametric tests. Statistical analyses were performed using SigmaStat 3.11.0 (Systat Software Inc., San Jose, CA) and with SAS v9.2 (SAS Institute, Cary, NC). Non-linear regressions were statistically assessed using the regression wizard in SigmaPlot 12 (Systat Software Inc., San Jose, CA).

Results

pH, base cations, ANC, and stream temperature

As intended by experimental design, all chemical features associated with acidity differed significantly ($P < 0.05$) among the study streams (Table 1). As pH declined from 6.98 ± 0.02 in Piney River to 5.34 ± 0.05 in Meadow Run, the sum of base cations decreased sequentially from 438 ± 16 to 109 ± 5 μEq l^{-1}, and ANC declined from 242.0 ± 7.8 to 4.5 ± 0.4 μEq l^{-1}. The charge sum of base cations, ANC, and pH differed concomitantly and were positively and highly correlated among streams ($r = 0.90$–0.98, $P = 0.037$–0.002, data not shown).

Average stream temperature ranged from 3.13 ± 0.06 to 6.84 ± 0.17°C (Table 1). Higher temperatures were generally found in more acidic streams (Online resource 2). ANC and stream temperature were negatively correlated ($r = -0.88$, $P = 0.047$). While not statistically significant, correlations between temperature and pH ($r = -0.73$, $P = 0.15$) and base cations ($r = -0.83$, $P = 0.08$) support the general trend for warmer temperatures in more acid-influenced streams.

Nutrients, DOC, litter, and fungal biomass

Stream water was depleted in all forms of measured nutrients. Concentrations of NH_4–N were consistently below detection and NO_3–N was scarce (0.9–2.4 μg l^{-1}, Table 1). Concentrations of both SRP (0.7–4.8 μg l^{-1}) and DOC (0.76–2.1 mg l^{-1}) were also low, but differed more extensively among streams than did NO_3^- (Table 1). Nutrients and DOC were positively correlated among streams ($r = 0.91$–0.98, Online Resource 3), but were not significantly related to pH, base cation concentrations, or ANC (data not shown).

Leaf litter stocks ranged from 173.9 to 293.2 g AFDM m^{-2} (Table 2), did not differ among streams (ANOVA, $P > 0.05$, Table 2), and were not related to any measure of stream acidity ($P > 0.05$). In contrast, average FB (mg OM g^{-1} AFDM) differed significantly among all streams (ANOVA, $P < 0.0001$), decreased sequentially from 37.7 ± 4.7 mg g^{-1} AFDM in the circumneutral stream to 2.9 ± 0.2 mg g^{-1} AFDM in the most acidic system (Table 2), and declined exponentially with decreasing

Table 2 Long-term pH, detrital stocks (mean ± SE), and N uptake parameters for the five study systems

	Meadow Run	Paine Run	Brokenback Run	Hazel River	Piney River
pH (long-term mean)	5.3	5.7	6.4	6.5	7.0
Detrital stocks					
Leaf standing stock (g AFDM m^{-2})[a]	$184.3^{A} \pm 38.9$	$193.3^{A} \pm 69.5$	$173.9^{A} \pm 44.9$	$293.1^{A} \pm 105.6$	$186.3^{A} \pm 51.7$
Leaf organic matter (%)	$95.8^{A} \pm 0.2$	$95.1^{AB} \pm 0.4$	$94.2^{CB} \pm 0.3$	$93.4^{C} \pm 0.5$	$94.5^{AB} \pm 0.4$
Fungal biomass (FB) (mg g^{-1} AFDM)[a]	$2.9^{A} \pm 0.2$	$4.6^{B} \pm 0.1$	$9.8^{C} \pm 2.1$	$11.8^{D} \pm 2.3$	$37.7^{E} \pm 4.7$
Fungal standing stock(FSS) (g m^{-2})[b]	0.54 ± 0.22	0.88 ± 0.55	1.70 ± 0.90	3.47 ± 2.45	7.03 ± 3.68
N uptake					
S_w (m)	254	319	287	502	305
v_f (mm min^{-1})	0.67	0.62	0.61	1.12	0.99
U (mg N m^{-2} d^{-1})	1.38	0.83	2.07	3.30	1.33

pH values are provided for comparative purposes

Mean values with different superscripts within a row are significantly different following significant 1-way ANOVA and Tukey's HSD among streams

[a]Statistical assessment was carried out on ln-transformed data

[b]Areal fungal standing stocks are presented with standard errors derived from error propagation and mean values are not amendable to statistical assessment

pH (Fig. 1a), reflecting a power-law relationship between FB and stream acidity (i.e., concentration of H^+). Similar litter abundance and non-linear decreases in FB (Fig. 1a) dictated differences in FSS (g OM m^{-2}) among streams. FSS decreased significantly and sequentially from 7.03 ± 3.68 (Piney River) to 0.54 ± 0.22 g OM m^{-2} (Meadow Run, Table 2), following a power-law decline with stream acidity (i.e., negative exponential relationship with pH, $P = 0.003$, Fig. 1b).

The trend for decreasing FB with decreasing pH is supported by multiple studies in Europe and the USA (Fig. 1c, d) specifically addressing fungal abundance in response to acidity derived from atmospheric deposition (i.e., without confounding influences of acid mine drainage). Across 10 studies of 55 streams and 73 sites quantifying ergosterol content of decaying leaves, absolute FB was not statistically related to ambient stream pH ($P > 0.05$, Fig. 1c). Following z-score transformation, however, FB declined significantly with decreasing pH (Fig. 1d). This relationship emphasizes consistent declines in response to increased acidity relative to mean abundance within local groups of streams. Normalized data reveal that declines are concomitant with larger-scale variation in other environmental variables that drive absolute abundance.

ER and qCO_{2E}

ER declined fivefold and non-linearly with decreasing FSS across the five study streams ($P = 0.004$, Fig. 2a). Moderate initial declines in ER were followed by steep decreases (5.2–1.1 g O_2 m^{-2} d^{-1}, Fig. 2a) as FSS dropped from 1.79 ± 0.90 to 0.54 ± 0.22 g OM m^{-2} (Table 2). Declining pH was linearly associated with decreased ER ($P = 0.004$, Fig. 2b), and ER was generally lower in warmer streams, but was not correlated with water temperature ($r = -0.63$, $P = 0.25$, Fig. 2c).

Whole-stream qCO_{2E} ranged from 0.39 to 1.4 g C g^{-1} C d^{-1}, and while generally greater in more acidic streams, it was not statistically related to any measure of acidity ($P > 0.05$, Online Resource 4). Temperature and ln-transformed qCO_{2E}, on the other hand, were significantly and positively related ($R^2 = 0.79$, $P = 0.042$, Fig. 3a). This relationship translated to a significant Arrhenius plot ($R^2 = 0.80$, $P = 0.041$, Fig. 3b) with a slope of − 2.3 ± 0.67 eV. The activation energy described by this plot suggests that the qCO_{2E} response across streams was in excess of that predicted by temperature alone (i.e., expected slope of − 0.6 eV).

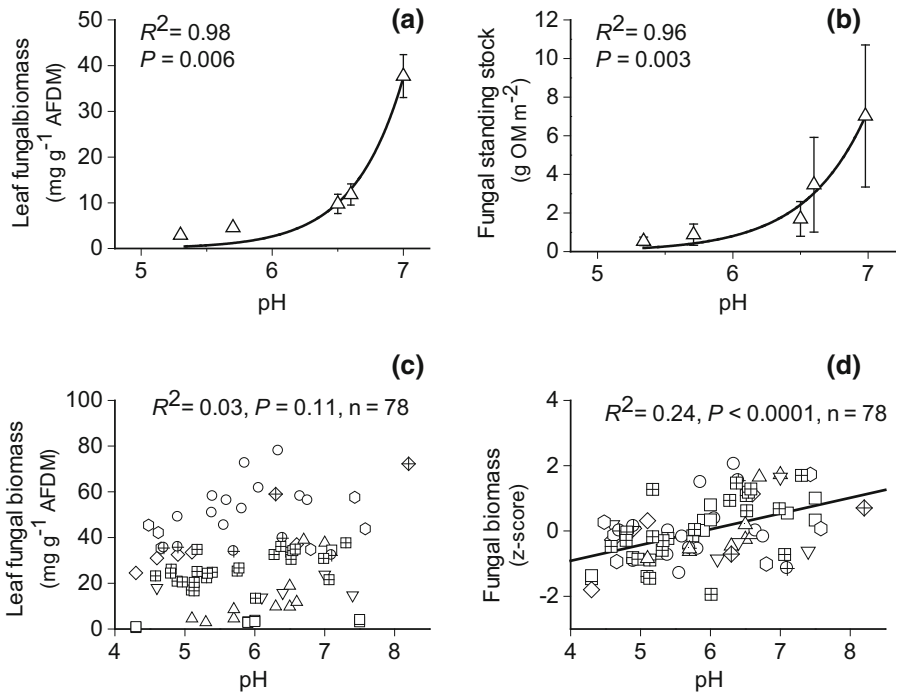

Fig. 1 Mean (± SE) fungal biomass per mass leaf material (**a**) and fungal standing stock (mean ± SE) (**b**) versus stream pH in the five study streams. Fungal biomass versus stream pH across 10 studies and 78 streams (**c**) in the USA and Europe where variation in stream acidification resulted from differential rates of atmospheric deposition and contrasting catchment geologic composition. Normalized fungal biomass (i.e., *z*-scores) versus pH for the same 78 streams (**d**). Symbols are triangles, current study; inverted triangles, Cornut et al. (2012); circle, Simon et al. (2009); square, Griffith & Perry (1994); diamond, Baudoin et al. (2008); hexagon, Clivot et al. (2013); circle crossed, Dangles & Chauvet (2003); square crossed, Dangles et al. (2004a); triangle dotted, Ely et al. (2010); diamond crossed, Methvin & Suberkropp (2003)

U_{NO_3}

Unlike other studies linking metabolic rates and nutrient cycling, no significant ($P > 0.05$) relationships existed among any measures of uptake (Sw, v_f, U_{NO_3}; Table 2) and ER or qCO_{2E} (data not shown). Areal uptake (U_{NO_3}, 0.83–3.30 mg N m^{-2} d^{-1}, Table 2) was not related to detrital or fungal abundance, nor to stream temperature, or any measure associated with stream acidity ($P > 0.05$). In contrast, U_{NO_3} increased with nutrient abundance (Fig. 4). As expected from Eq. (3), U_{NO_3} increased with NO_3–N concentrations (R^2 = 0.79, P = 0.043, log–log plot, Fig. 4a). Areal uptake also increased log-linearly with increasing SRP (Fig. 4b, R^2 = 0.77, P = 0.048), and linearly with DOC (R^2 = 0.82, P = 0.039, Fig. 4c).

Discussion

Emergent behavior of ecosystems across a stress gradient reflects interaction among stressors (Townsend et al., 2008; Omerod et al., 2010; Matthaei et al., 2010; Piggott et al., 2015b) and other intrinsic and extrinsic features driving metabolism (Valett et al., 2008; Huryn et al., 2014) and material processing (Jenkins et al., 2013). In this study, we assessed acidification, a known stressor in aquatic systems (Driscoll et al., 2001), to show how it may interact with extant constraints like temperature (Ferreira & Chauvet, 2011) to influence physiological mechanisms and produce emergent responses at the landscape scale. In many ways, the alterations to ecosystem structure and function reported here fall within those expected for stream ecosystems experiencing the 'acidification syndrome' described by Ferreira & Geurold (2017). With this perspective, response to acidification potentially includes

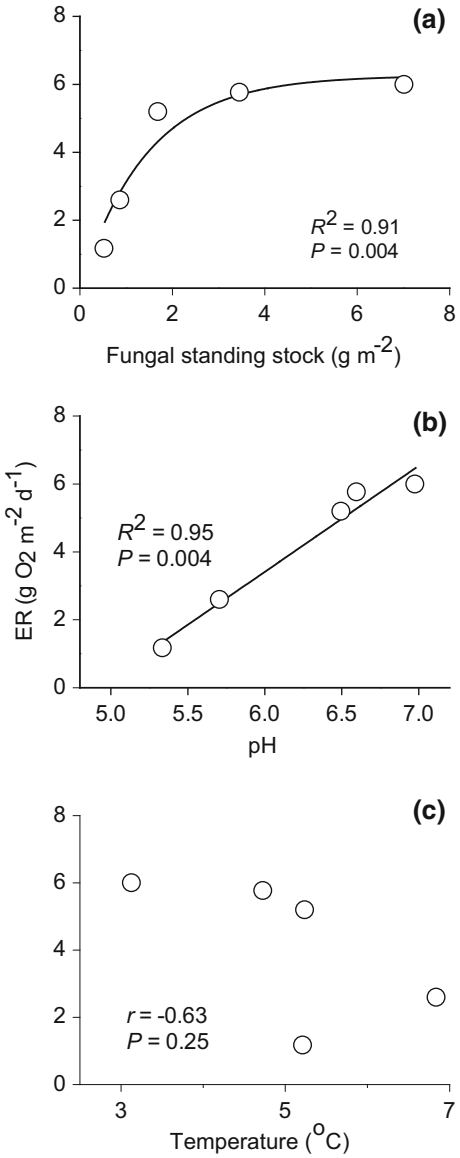

Fig. 2 Rates of ecosystem respiration (ER) versus **a** fungal standing stock, **b** stream water pH, and **c** stream water temperature

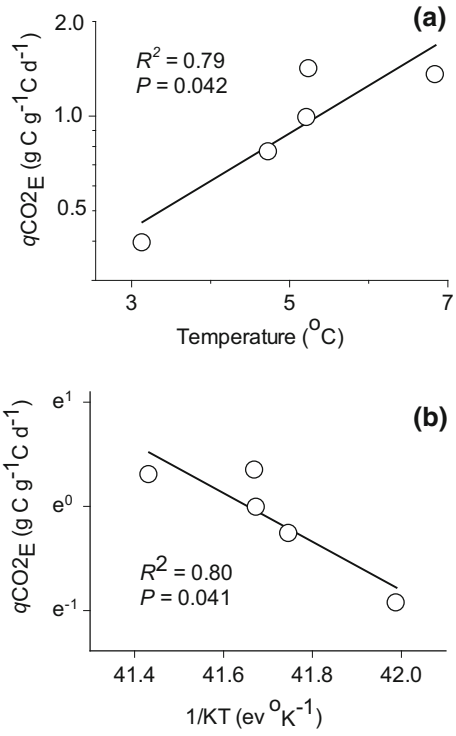

Fig. 3 Ecosystem metabolic quotient (qCO_{2E}) versus **a** stream temperature and **b** temperature related through an Arrhenius scaling relationship where Ln (qCO_{2E}) = − 2.33 (1/KT)–97.1

decreases in fungal abundance, species richness, and microbial respiration, altered community composition and enzyme activity, and lowered abundance and activity of macroinvertebrate detritivores, culminating in decreased rates of litter decomposition. Which features of the syndrome are encountered, and the relative magnitudes of their influences, are likely tied to interactions among features of acidification, other extant stressors, and existing environmental constraints.

ER, litter, and fungal abundance

Litter abundance did not differ significantly among our streams and was typical of headwaters in deciduous forests, representing 61–104% of maximal stocks measured over a 5-year period in two streams of the Coweeta Hydrologic Laboratory, NC, USA (Suberkropp et al., 2010). While our study streams contained similar amounts of leaf detritus, ER declined strikingly across streams, reflecting an evident loss of FB per unit leaf mass with decreasing stream pH (Fig. 1a). Fungi dominate microbial biomass and activity during leaf decomposition in streams (Gessner & Chauvet, 1994; Gulis & Suberkropp, 2003; Pascoal & Cassio, 2004) and a broad base of research addressing soil systems indicates that fungi are proportionally dominant compared to bacteria as pH declines (Blagodatskaya & Anderson, 1998; Bååth & Anderson, 2003; Rousk et al.,

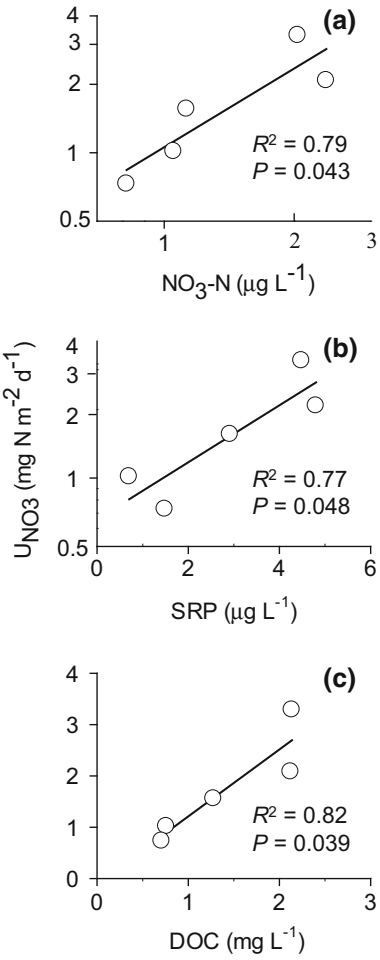

Fig. 4 Areal N uptake (U_{NO_3}) versus stream water concentrations of **a** nitrate nitrogen (NO_3–N), **b** soluble reactive phosphorus (SRP), and **c** dissolved organic carbon (DOC)

2009, 2010a, b). Our contention that fungal decline is responsible for decreases in respiration is supported by the fact that 91% of the variance in ER encountered across our study streams was explained by fungal abundance (Fig. 2a), suggesting little residual variance associated with other respiratory biota.

One year prior to this investigation, we observed comparable maxima and minima for FB, and a similar associated exponential decline along an acidity gradient in SNP that included some of the same streams used in this study (Ely et al., 2010). Absolute declines in FB observed in our studies, however, are drastic compared to others related to stream acidification (Fig. 1c). The very low fungal biomass found in our most acidic streams likely reflects the combined influences of increased acidity, generally low temperatures, and sparse nutrient availability.

In any case, normalizing spatial trends in fungal abundance to mean values within studies (i.e., z-score calculation) illustrates the tendency for relative fungal mass to decline with increased acidity derived from atmospheric deposition. Metal mobilization associated with acid mine drainage (Niyogi et al., 2001, 2002) and differing nutrient concentrations (Methvin & Suberkropp, 2003) may alter fungal abundance for any given pH, in part explaining the broad range in observed responses to acidification.

Ecosystem metabolic quotient (qCO_{2E})

The strong relationship between FB and ER (Fig. 2a) and the significant correlation relating qCO_{2E} and temperature (Fig. 3) suggest that qCO_{2E} retains value as an ecosystem-level metric useful for comparisons among streams receiving autumnal leaf litter loads. Increasing qCO_2 reflects decreasing metabolic efficiency as a response to potential stressors, historically including enhanced acidity (Wardle & Ghani, 1995). Whole-system assessment across our acidity gradient revealed a trend for increased qCO_{2E} in more acidic systems, but relationships between qCO_{2E} and measures of acidity were weak and not statistically significant (Online Resource 4). These results differ from our earlier study using microcosms where qCO_{2E} increased with acidity and was tightly correlated to biomass-specific N uptake in the presence of excess N (Ely et al., 2010). More complicated responses at the whole-system level of assessment likely reflect interactions among acidity and concomitant environmental influences (e.g., temperature, nutrient availability) purposefully avoided in our microcosm experiments.

qCO_{2E} among SNP study streams was positively correlated to stream temperature, but closer assessment reveals an atypical relationship. Arrhenius scaling between ER and temperature should yield the canonical value of 0.65 eV applicable to all ecosystems (Brown et al., 2004), but the slope observed for our streams was more than threefold greater. Cross et al. (2015) recently emphasized that Arrhenius assessment of field data represents 'apparent activation energy' that may reflect covariance between temperature, nutrients, and other external influences. Piggott et al. (2015a) refer to reinforcement among multiple stressors as 'synergism' such that they result

in cumulative effects that are at least additive, but potentially greater than the sum of the influences of the individual stressors. In our study, the potential for additive effects is illustrated by the enhanced response to temperature displayed by qCO_{2E} that may reflect the influence of acidity given the positive relationship between temperature and acidity observed across streams (Online Resource 2).

Others have identified interaction with temperature as central to understanding aquatic responses to acidification. At the organismal level, covariance between acidity and temperature represents a homeostatic challenge to other poikilotherms like marine fishes stressed by ocean acidification (Munday et al., 2009). In terms of ecosystem processes, Hildrew et al. (1984) and Jenkins et al. (2013) showed that temperature and stream acidity interacted to influence rates of decomposition with the potential for temperature effects to change among seasons from reinforcing to countering.

Nitrogen uptake—U_{NO_3}

Nutrient uptake is closely related to availability in freshwater ecosystems, and in our study NO_3–N concentrations were low and generally similar among streams (Table 1) resulting in lower uptake rates than reported by others using ^{15}N (e.g., Mulholland et al., 2008). Whole-stream uptake did not respond consistently to stream acidity, but increased with greater concentrations of N, P, and organic C (Fig. 4), indicating sensitivity to multiple dissolved resource pools and restricted N sequestration under conditions of nutrient limitation. At the same time, U_{NO_3} was not related to ER or qCO_{2E}, despite documented close coupling of nutrient uptake and metabolic activity in streams (Huryn et al., 2014), lakes (Sterner & Elser, 2002), and oceans (Smith & Hollibaugh, 1997). The decoupling of metabolism and nutrient uptake from the water column described here may reflect the very low N in the water column and reliance on N associated with detrital substrates (Cheever et al., 2012; Cheever & Webster, 2014). While Mulholland et al. (1985) showed that P uptake was related to leaf biomass during autumn, Pastor et al. (2014) emphasized that leaf biofilm reliance on stream water N changed over the course of decomposition. Thus, the relationships observed between FB and U_{NO_3} may be reflect in-stream influences of acidity and nutrient availability, and the time-course of biotic resource utilization associated with litter decomposition.

Systems assessment of stress and concomitant drivers

Ecosystem responses to acidity may depend on the sources and origins of acidity (i.e., natural versus human-induced). Despite several studies reporting the negative influences of human-induced acidification, boreal streams of northern Sweden are naturally acidic and do not display the reduced biotic diversity or lower processing rates characteristic of high-elevation streams exposed to acidification via atmospheric deposition (Dangles et al., 2004b). This suggests that stress associated with acidification of running waters depends on the extent to which biota are adapted to chronic acidification alone, or by a combination of acidification and other stressors observed in human-influenced streams over shorter time frames. Our study focuses on how stress from anthropogenic acidification influences the coupling between microbial activity and ecosystem function. Results suggest that stressors occur simultaneously with other exogenous factors to influence key feedback loops linking biota and resources, resulting in collective system behavior. Here we emphasize that this co-occurrence is fundamental to understanding natural systems exposed to increasingly influential environmental stressors. Modern environmental scenarios, therefore, necessitate concomitant assessment in order to manage multiple influences under conditions of global change.

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