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# Effects of stream phosphorus levels on microbial respiration

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## SUMMARY

1. We examined microbial respiration among streams in lowland Costa Rica comprising a natural phosphorus gradient (5–350 μg SRP $L^{-1}$) resulting from variable inputs of solute-rich (e.g. P, $SO_4$ and Cl) groundwater.
2. Microbial respiration rates were determined by measuring oxygen change *in situ* in nine low-order streams on three substrate types: mixed leaves collected from the stream bottom, conditioned *Ficus* leaves and sediments.
3. Respiration rates on both leaf types were positively related to phosphorus and negatively related to N : P ratios. Microbial respiration rates on sediments were not related to any of the variables [i.e. soluble reactive phosphorus (SRP), N-$NO_3$ and N : P] measured.
4. Respiration rates on newly colonised *Ficus* leaves formed an asymptotic curve increasing to a plateau, suggesting that saturation with phosphorus occurred at concentrations <15 μg SRP $L^{-1}$.
5. To test the hypothesis that phosphorus was the main solute in solute-rich water that was driving observed differences in microbial respiration rates, we artificially enriched a small stream with phosphorus and measured changes in respiration before and after enrichment.
6. Experimental phosphorus enrichment produced increases in respiration rates similar in magnitude to those observed in the nine streams forming the natural phosphorus gradient, supporting our hypothesis that phosphorus was the major variable driving interstream differences in microbial respiration rates. Respiration rates were higher in this study than those reported for most other tropical streams and rivers with the exception of those reported for tropical Asian streams.
7. Results indicate that variations in phosphorus concentrations can potentially affect patterns of microbial respiration rates at a landscape level via differential inputs of solute-rich groundwater into streams.

*Keywords*: enrichment, geothermal inputs, microbial respiration, phosphorus, tropical streams

## Introduction

A major challenge for ecologists is to determine how solutes in groundwater can influence biological patterns in surface waters (Pringle & Triska, 2000). In tectonically active areas, geothermal activity can dramatically modify the chemical characteristics of groundwater. When groundwater mixes with surface water it can introduce variability in the chemical composition of streams. Geothermally modified groundwater is often characterised by high solute concentrations (e.g. Na, Ca and Mg) and has been classified into different groundwater 'types' based on

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pH and the dominant cation or anion (White, 1957; Henley, 1985). In Central America, streams draining volcanic landscapes have wide ranges in solute composition, because of variable inputs of geothermally modified groundwater (Pringle *et al.*, 1993). In the foothills of Costa Rica's Cordillera Central at La Selva Biological Station, geothermally modified water is classified as a 'dilute sodium chloride bicarbonate' type and it is rich in phosphorus (Pringle *et al.*, 1993).

In this study we hypothesised that interstream differences in solute concentrations, caused by variation in geothermally modified groundwater inputs, would result in differences in microbial activity among streams draining La Selva Biological Station. We measured respiration rates because they are considered to be good indicators of microbial activity within an ecosystem (Fuss & Smock, 1996; Hill, Herlihy & Kaufmann, 2002): microbes (i.e. fungi and bacteria) are important components of stream ecosystems, as they are the basal trophic level in heterotrophic food webs (Allan, 1995). As previous studies at La Selva identified phosphorus as a limiting nutrient to microbially mediated rates of decomposition (e.g. Rosemond *et al.*, 2002) and algal growth (Pringle & Triska, 1991), we further hypothesised that, of all solutes in geothermally modified groundwater, phosphorus would be the main driver of potential patterns in microbial respiration rates. To test our hypotheses we measured microbial respiration rates in nine streams representing a gradient of phosphorus (5–350 μg SRP $L^{-1}$). We also experimentally enriched a small stream with phosphorus and measured changes in respiration before and after enrichment.

## Methods

### Study area

This study was conducted at La Selva Biological Station (10°26′N, 84°01′W) in Costa Rica. The 1536-ha reserve is near sea level (35–150 m a.s.l.) on the Caribbean slope and adjacent to the Braulio Carrillo National Park. La Selva is a tropical wet forest that receives almost 4000 mm of rainfall a year, with >400 mm $month^{-1}$ from May to December (Sanford *et al.*, 1994). Stream water temperature is relatively constant throughout the year (range 24–27 °C), with mean annual pH values ranging from 5 to 6 (Ramírez, 2001).

Streams at La Selva are naturally variable in their solute concentrations because of differential inputs of geothermally modified groundwater (Pringle, 1991). Volatile gases from a deep magma source are absorbed into groundwater at high elevations. This geothermally modified groundwater cools and further changes its chemical composition as it weathers rocks during its flow downslope, where it eventually enters lowland streams. One of the main signatures of this modified groundwater is high solute concentrations (i.e. up to 350 μg SRP $L^{-1}$, 28 mg Cl $L^{-1}$, 13 mg $SO_4$ $L^{-1}$, 19 mg Ca $L^{-1}$, 44 mg Na $L^{-1}$, 25 mg Mg $L^{-1}$) relative to unmodified groundwater (i.e. <10 μg SRP $L^{-1}$, <3 mg Cl $L^{-1}$, <2 mg $SO_4$ $L^{-1}$, <2 mg Ca $L^{-1}$, <3 mg Na $L^{-1}$, <1 mg Mg $L^{-1}$; Pringle *et al.*, 1993). As a result, streams at La Selva form a mosaic of solute-poor and solute-rich systems, which are in close proximity to each other (Pringle, Triska & Browder, 1990; Pringle, 1991). In contrast to phosphorus and the other solutes listed above, nitrate does not differ significantly between geothermally modified and unmodified streams (Pringle, 1991). Dense riparian cover in most streams (canopy cover >80%) results in light-limited algal assemblages (Pringle *et al.*, 1986) and detritus-based food webs.

### Stream survey

Solute concentrations and microbial respiration were measured in eight low-order streams during July 1998. Streams were selected to form a gradient in solute concentrations, based on ongoing research (C.M. Pringle, unpublished data). Streams ranged from solute poor, with low soluble reactive phosphorus (SRP) concentrations and no inputs of geothermally modified groundwater, to solute rich, with high SRP concentrations and significant inputs of geothermally modified groundwater. We added a ninth stream, the Taconazo, in July 1999 in order to include an additional solute-poor stream. All sites were surrounded by primary forest and canopy cover was ~85%. Benthic substrates were composed mainly of fine sediments and leaf accumulations in runs and pools, but boulders were present in some reaches.

Nutrient concentrations were measured by collecting two filtered (0.45 μm Millipore filters) water samples at each stream. Samples were frozen until analysis at the University of Georgia, U.S.A. Nitrate ($NO_3$-N), ammonium ($NH_4$-N) and phosphorus (as

SRP) levels were measured using continuous-flow colorimetry and an Alpkem RFA 300 colorimetric analyzer (OI Corporation, College Station, TX, USA). The cadmium reduction, phenate and ascorbic acid methods were used for nitrate, ammonium and SRP analyses, respectively (APHA, 1992). Concentrations of $SO_4$, Ca, Na, Mg and K in each stream were measured in one of the water samples collected for nutrients, using ion chromatography (APHA, 1992). Atomic N : P ratios were calculated using $NO_3$-N plus NH4-N and SRP. Temperature and pH were measured using a pH-meter (Hanna Instruments, RI, USA).

Respiration measurements were made using custom-made metabolism chambers (Plexiglass boxes mounted on fibreglass bases, constructed by Rapid Creek Research, Boise, Idaho). Each chamber was 12 × 5 cm in cross-section and was equipped with a pump that produced recirculating flow velocities of 2.4 cm $s^{-1}$ through the chamber. Chambers were filled with stream water, anchored to the stream bottom to maintain ambient stream temperatures, and covered with a black plastic sheet to inhibit photosynthesis. Microbial respiration rates on each substrate type were measured using four chambers (replicates, $n = 4$ per stream). Because of rapid oxygen depletion inside chambers, respiration was measured as the change in oxygen concentration in 1 h. Oxygen was measured using YSI Model 58 dissolved oxygen meters (YSI Inc., Yellow Springs, OH, USA). Percentage of nitrogen (%N) and the carbon-to-nitrogen (C : N) ratios in each sample used in respiration chambers were estimated by collecting a subsample and using a Carlo Erba NA 1500 CHN Combustion Analyzer (Carlo Erba, Milan, Italy).

Microbial respiration rates were measured on: (1) mixed leaves from the stream bottom, (2) sediments (the dominant stream bottom substrate) and (3) 8-day *in situ* preconditioned *Ficus insipida* Willd. leaves. Respiration on mixed leaves was measured using leaves randomly collected from the stream bottom. Invertebrates and sediments trapped among leaves, but not incorporated into the biofilm, were removed manually before placing leaf packs in chambers. Sediment samples for respiration measurements were placed in plastic containers 8 days before measurements were made in chambers. Sediments from the top 15 cm of stream bottom were carefully cored to avoid mixing and used to fill the containers (10 cm diameter and 6 cm depth), making sure that layers remained in the same order as when collected (i.e. sediments in contact with surface water were at the top of the containers). Leaves of *F. insipida,* a dominant riparian tree in lowland Central America (Hartshorn, 1983), served as a standard substrate among streams to control for variability in microbial colonisation of leaves and spatial variability in microbial biomass in the streams. Groups of *Ficus* leaves (~5 g dry mass each) tethered with binder clips were conditioned in each stream for 8 days before measuring respiration rates. Previous studies indicated that 8 days provided enough exposure to ensure high microbial colonisation (Rosemond *et al.*, 2002; A. Ramírez, unpublished data). As with mixed leaves, invertebrates and sediments were removed by hand before incubation in metabolism chambers.

Microbial respiration rates were expressed as oxygen consumed by microbes per gram ash-free dry mass (AFDM) per hour (mg $O_2$ $g^{-1}$ AFDM $h^{-1}$). To allow comparisons with other studies, we estimated areal respiration (per $m^2$ of stream bed) by weighting respiration rates of mixed leaves and sediments by their proportional contribution to total organic matter in the stream channel. The proportional contribution of each substrate was determined by visually assessing a 50-m reach and estimating the area covered by leaves and sediments. Estimates were done independently by three people and means were used in the calculations. Particulate organic matter content of each substrate type was determined by collecting three core samples (area: 0.006 $m^2$ each). Areal respiration rates were also expressed in carbon units (kg C $m^{-2}$ $year^{-1}$) using a factor of 2.75 to convert oxygen to carbon (Winberg, 1971).

### *Phosphorus enrichment*

We conducted a whole-stream phosphorus enrichment experiment in the Carapa, a first-order stream draining into the Sura. The enrichment was initiated in July 1998 (Ramírez, 2001) and has been continuously maintained ever since. We added phosphoric acid to increase phosphorus concentrations from background levels of <5 to ~200 μg SRP $L^{-1}$, a mean concentration for streams receiving geothermally modified groundwater inputs at La Selva (e.g. Pringle, 1991). A Mariotte bottle was used to continuously add phosphoric acid using the procedures described by Pringle & Triska (1997) for whole-stream manipulations. The concentration of phosphoric acid in the bottle and the addition rates were adjusted based on stream discharge to maintain SRP enrichment near target concentration. Nitrate ($NO_3$-N) and SRP concentrations were measured before and after the addition of phosphorus using methods described above. Effects of phosphoric acid addition on stream pH were assessed during January 2000 by measuring pH 2 m above and 10 m below the point of enrichment. At each location, pH was measured in nine random points and at different depths.

For the present study we measured microbial respiration rates on mixed leaves and on *Ficus* leaves 2 weeks before and 2 weeks after the beginning of the enrichment. On each occasion, microbial respiration rates were measured on both substrates on three different days (replicates, $n = 3$) using four chambers per substrate type. Measurements were conducted as described above.

### *Statistical analysis*

Surveyed streams were ordered along a solute concentration gradient using principal component analysis (PCA). The PCA was run using PC-ORD for Windows, version 3.0 (McCune & Mefford, 1997). We ordinated all water column solute concentrations, excluding N : P ratios, for each stream (i.e. SRP, $NO_3$-N, $NH_4$-N, Cl, $SO_4$, Ca, Na, Mg and K) using untransformed data. We used the correlation cross-products matrix to produce a standardised PCA in which values were centred and standardised by the standard deviation.

Microbial respiration rates at all surveyed streams were correlated against PCA axis 1 and 2 scores to assess overall trends in respiration on the stream solute gradient. In addition, microbial respiration rates were regressed against water column nutrient concentrations (i.e. SRP, $NO_3$-N and N : P) and substrate %N. The relationships between microbial respiration rates and SRP were non-linear; therefore, they were fitted to a Michaelis–Menten model of enzyme-catalysed reactions (Stryer, 1981). The model has the following form:

$$V = V_{\max}([S]/([S] + K_m))$$

where $V$ is the rate of catalysis of an enzyme, [S] is the substrate concentration, $V_{\max}$ is the maximum rate of catalysis, and $K_m$ is the substrate concentration at which the reaction is half the maximum value. This model appeared to be a good descriptor of the relationship between SRP concentration ([S]) and microbial respiration rates ($V$). The model was fitted to untransformed data using the iterative, least-squares nonlinear fitting function in JMP (Version 4.0.4, SAS Institute, Cary, NC, U.S.A.).

The effect of phosphorus enrichment on pH and microbial respiration rates in the Carapa whole-stream enrichment experiment was tested using a *t*-test. The results of this experiment should be interpreted with caution, as the experiment was not replicated (i.e. we used only one stream). All analyses, except PCA, were run in JMP (SAS Institute).

## Results

Stream solute concentrations were very different among the streams surveyed (Table 1). All solutes measured formed a concentration gradient, with Taconazo having the lowest solute concentrations and Saltito-60, Sura-30 and Arboleda the highest (Table 1).

**Table 1** Characteristics of the study streams ordered by increasing concentration of soluble reactive phosphorus

| | Conductivity (μS) | pH | SRP (μg L$^{-1}$) | $NO_3$ (μg L$^{-1}$) | $NH_4$ (μg L$^{-1}$) | N : P | $SO_4$ (mg L$^{-1}$) | Ca (mg L$^{-1}$) | Na (mg L$^{-1}$) | Mg (mg L$^{-1}$) | K (mg L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Taconazo | 23 | 4.2 | 4.7 | 176.6 | 68.7 | 115.6 | 1.5 | 1.2 | 1.5 | 0.5 | 0.6 |
| 2. Carapa | 21 | 4.3 | 10.2 | 184.3 | 20.7 | 44.5 | 1.0 | 0.9 | 1.4 | 0.4 | 0.7 |
| 3. Saltito-100 | 16 | 4.3 | 11.2 | 148.5 | 30.6 | 35.6 | 1.0 | 1.0 | 1.5 | 0.5 | 0.6 |
| 4. Piper | 23 | 4.5 | 12.4 | 183.3 | 130.5 | 56.0 | 1.0 | 1.8 | 1.7 | 0.8 | 0.5 |
| 5. Sura-60 | 23 | 4.3 | 12.5 | 225.3 | 15.8 | 42.7 | 0.9 | 1.2 | 1.6 | 0.6 | 0.7 |
| 6. Salto-60 | 28 | 5.2 | 26.6 | 262.9 | 9.8 | 22.7 | 1.0 | 1.7 | 2.2 | 0.8 | 1.1 |
| 7. Saltito-60 | 88 | 5.9 | 78.7 | 164.0 | 20.9 | 5.2 | 1.7 | 4.9 | 5.7 | 4.3 | 1.5 |
| 8. Sura-30 | 158 | 5.9 | 226.8 | 201.9 | 11.1 | 2.1 | 2.1 | 9.7 | 9.6 | 7.6 | 2.6 |
| 9. Arboleda | 297 | 5.9 | 351.5 | 210.5 | 14.8 | 1.4 | 3.9 | 17.4 | 18.5 | 15.5 | 4.6 |

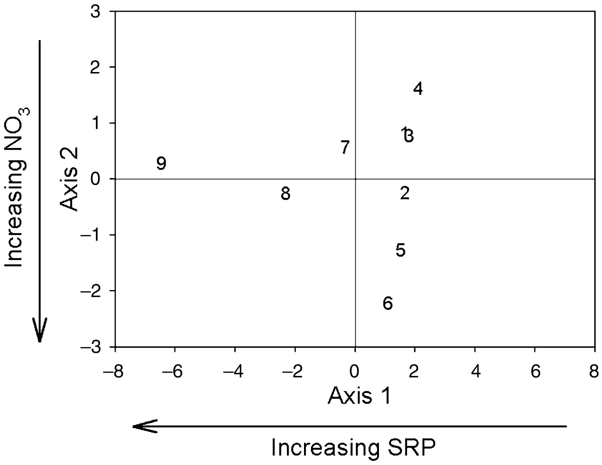

**Fig. 1** Ordination of the study streams based on solute concentrations using Principal Component Analysis (PCA). The streams are (1) Taconazo, (2) Carapa, (3) Saltito-100, (4) Piper, (5) Sura-60, (6) Salto-60, (7) Saltito-60, (8) Sura-30 and (9) Arboleda.

There were no differences in stream temperature (range 25–26 °C). Values of pH ranged from 4.2 in low solute streams to 5.9 in high solute streams (Table 1).

The PCA ordination of stream solutes revealed that streams formed two main gradients (Fig. 1). Along axis 1, streams receiving inputs of geothermally modified groundwater had negative scores (i.e. Arboleda, Sura-30 and Saltito-60), whereas streams without inputs had a positive score (Taconazo, Sura-60, Salto-60, Piper, Carapa, Saltito-100; Fig. 1). Axis 1 was negatively correlated with SRP concentrations ($r^2 = 0.98$). Streams also formed a gradient along axis 2, which was negatively correlated to $NO_3$-N concentrations ($r^2 = 0.70$). The PCA axis 1 explained 78% of the variation and axis 2 explained an additional 13%.

Microbial respiration rates were highest on *Ficus* leaves (mean: 1.91 mg $O_2$ $g^{-1}$ AFDM $h^{-1}$; range: 1.27–2.49) and lowest on sediments (mean: 0.01 mg $O_2$ $g^{-1}$ AFDM $h^{-1}$; range: 0.004–0.016; Table 2). Mean areal respiration was 24.0 g $O_2$ $m^{-2}$ $day^{-1}$ and ranged from 1.9 to 67.9 (Table 2). Areal rates in carbon units ranged from 0.22 to 7.90 kg C $m^{-2}$ $year^{-1}$ (Table 2).

Microbial respiration rates on all substrates were negatively correlated with PCA scores on axis 1, indicating that streams with high SRP concentrations also had high microbial respiration rates. Only respiration on sediments was correlated with axis 2 and the relation was positive: streams with low $NO_3$-N concentration had high sediment respiration rates. However, $NO_3$-N concentrations were not significantly related to microbial respiration rates on any of the organic leaf substrates studied (Table 3). In contrast, microbial respiration rates on *Ficus* leaves were positively related to concentrations of SRP (Fig. 2a) and to %N of the leaves, and they were negatively related to water column N : P ratios (Table 3, Fig. 3a). The relationship between microbial respiration and SRP concentrations for *Ficus* leaves was not linear: respiration rates increased only within a narrow range of SRP concentrations (between 5 and 27 μg SRP $L^{-1}$), after which no further increase was observed (Fig. 2a). A similar pattern was found for the relationship between SRP and respiration rates on mixed leaves; however, it was not significant (Fig. 2b). Half saturation con-

**Table 2** Respiration rates (mg $O_2$ $g^{-1}$ AFDM $h^{-1}$) on the three substrate types studied and areal rates. Values are means of four measurements ± one standard error (SE), ordered by increasing SRP concentration, see Table 1

| | *Ficus* leaves | | Mixed leaves | | Sediments | | Areal rates | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | g $O_2$ $m^{-2}$ $day^{-1}$ | kg C $m^{-2}$ $year^{-1}$ |
| Taconazo | 1.267 | 0.101 | 0.669 | 0.014 | – | – | – | – |
| Carapa | 1.490 | 0.377 | 0.371 | 0.026 | 0.004 | 0.001 | 23.498 | 2.734 |
| Saltito-100 | 1.809 | 0.177 | 1.133 | 0.139 | 0.007 | 0.001 | 3.378 | 0.393 |
| Piper | 1.985 | 0.075 | 0.620 | 0.070 | 0.016 | 0.003 | 2.432 | 0.283 |
| Sura-60 | 2.068 | 0.334 | 0.779 | 0.043 | 0.007 | 0.003 | 8.021 | 0.933 |
| Salto-60 | 2.281 | 0.125 | 0.970 | 0.264 | 0.006 | 0.003 | 1.919 | 0.223 |
| Saltito-60 | 1.945 | 0.153 | 1.782 | 0.212 | 0.010 | 0.002 | 66.597 | 7.748 |
| Sura-30 | 1.904 | 0.224 | 0.791 | 0.158 | 0.006 | 0.002 | 17.755 | 2.066 |
| Arboleda | 2.488 | 0.391 | 1.246 | 0.045 | 0.015 | 0.002 | 67.914 | 7.901 |
| Mean | 1.915 | | 0.929 | | 0.010 | | 24.002 | 2.792 |

**Table 3** Regression coefficients ($r^2$) relating microbial respiration rates on *Ficus* and mixed leaves to nutrient concentration in the water (SRP, $NO_3$ and N : P) and in the substrate (%N)

| | *Ficus* leaves | | Mixed leaves | |
|---|---|---|---|---|
| | $r^2$ | $P$ | $r^2$ | $P$ |
| Phosphorus (SRP) | 0.72 | 0.004 | 0.10 | NS |
| $NO_3$ | 0.32 | NS | 0.02 | NS |
| N : P in water | 0.59 | 0.01 | 0.52 | 0.02 |
| %N in substrate | 0.47 | 0.05 | 0.38 | NS |

NS = not significant.

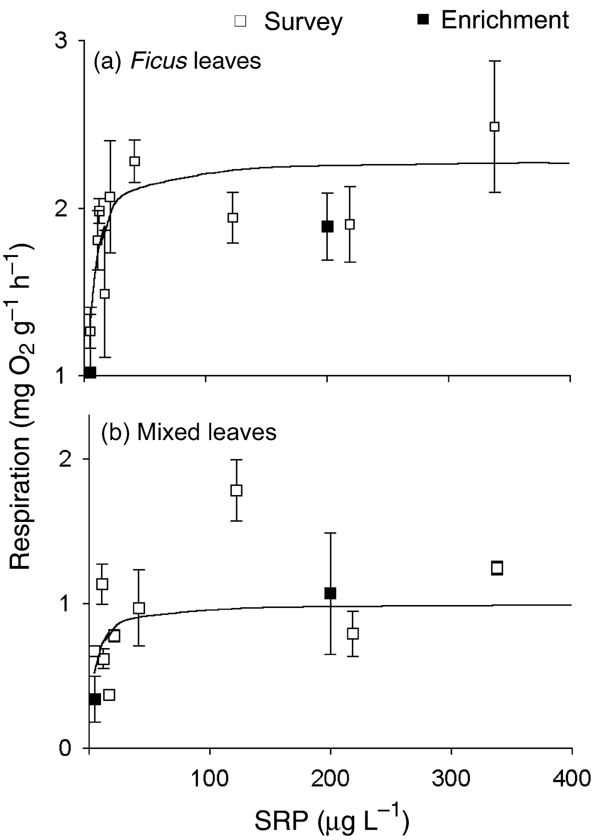

**Fig. 2** Relationship between phosphorus (SRP) concentrations and microbial respiration rates on (a) *Ficus* leaves and (b) mixed leaves. Open squares are mean respiration rates ($n = 4$) measured in the field and solid squares are mean respiration rates ($n = 3$) measured in the phosphorus enrichment experiment. Equation of the line (i.e. regression, Table 3) for '*a*' is 1/Respiration = 0.436 + 1.538 × 1/SRP, and '*b*' is 1/Respiration = 0.999 + 4.267 × 1/SRP. Equations include data from the survey only. See Table 3 for $r^2$ and $P$-values.

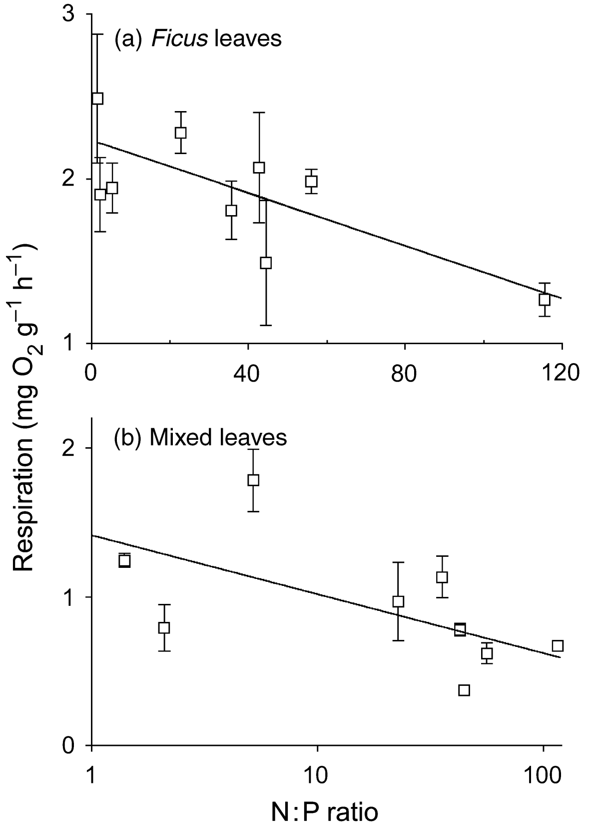

**Fig. 3** Relationship between N : P ratios and microbial respiration rates on (a) *Ficus* leaves and (b) on mixed leaves. Squares are mean respiration rates ($n = 4$) measured in the field. Equation of the line (see Table 3) for '*a*' is Respiration = 2.234 − 0.008 × SRP and for '*b*' is Respiration = 1.623 − 0.223 × $\text{Log}_{10}$ (SRP). See Table 3 for $r^2$ and $P$-values.

stants ($K_m$) from the Michaelis–Menten model were 2.87 and 6.96 µg SRP $L^{-1}$ for *Ficus* and mixed leaves, respectively. Exclusion of the three high phosphorus streams from the relationship in Fig. 2 resulted in a significant linear regression between phosphorus concentration and microbial respiration rates on *Ficus* leaves ($r^2 = 0.71$, $P = 0.03$), but not on mixed leaves ($r^2 = 0.17$, $P > 0.05$). Microbial respiration rates on mixed leaves were positively related to %N of the leaves and negatively related to water column N : P ratios (Table 3). However, the relationship was significant only for N : P ratios (Table 3, Fig. 3b). Microbial respiration rates on sediments were more variable among streams, and no clear relationships were observed between respiration rates and nutrient concentrations. Although streams differed in their water pH values, regression analysis showed that microbial respiration rates on the studied substrates were not related to water pH ($P > 0.05$ for all substrates).

Percentage of nitrogen was lower in sediments (mean: 0.24%) than in leaves (means: 1.87 and 1.93% for preconditioned *Ficus* and mixed leaves, respectively). Overall, *Ficus* and mixed leaves did not differ significantly in %N (*t*-test, d.f. = 59, $P = 0.30$); however, they differed in their C : N ratios. ANOVA indicated that *Ficus* had significantly lower C : N ratios (mean: 20.4) than mixed leaves (mean: 23.3) and that C : N ratios of *Ficus* and mixed leaves were marginally different among streams (ANOVA, Stream: d.f. = 7, $F = 2.19$, $P = 0.05$; Leaf type: d.f. = 1, $F = 14.4$, $P < 0.001$; Interaction: d.f. = 7, $F = 1.0$, $P = 0.42$). In addition, coefficients of variation (i.e. SD divided by the mean) were significantly higher for mixed leaves (mean: 14.6%) than for *Ficus* (mean: 6.8%; ANOVA, Leaf type: d.f. = 1, $F = 6.02$, $P = 0.02$).

The whole-stream phosphorus enrichment of Carapa effectively increased SRP concentrations from a background of <5 to ~200 μg $L^{-1}$ (range 150–250 μg $L^{-1}$). Stream water pH significantly decreased in the enriched segment by 0.10 pH units, mean pH upstream was 4.86 and decreased to 4.76 downstream of the enrichment point (*t*-test, d.f. = 16, $P = 0.03$). Microbial respiration rates on *Ficus* leaves showed a significant increase from a mean value of 1.0–1.9 mg $O_2$ $g^{-1}$ AFDM $h^{-1}$ (*t*-test, d.f. = 4, $P = 0.02$), while respiration rates on mixed leaves increased from 0.3 to 1.0 mg $O_2$ $g^{-1}$ AFDM $h^{-1}$ (*t*-test, d.f. = 4, $P = 0.05$), before and after stream enrichment. Increases in respiration rates after phosphorus enrichment in both leaf types were of similar magnitude to those observed along the phosphorus gradient (Fig. 2).

## Discussion

Results support our initial hypothesis that variations in water solute concentration (i.e. resulting from geothermally modified groundwater) result in differences in microbial respiration rates among streams. We found that streams with inputs of geothermally modified groundwater were richer in several solutes, including phosphorus, and had higher respiration rates than non-geothermal streams. While streams with no inputs of geothermally modified groundwater had low solute concentrations and differed mainly in their nitrate concentrations, differences in nitrate were not related to respiration rates. As both kinds of streams are common in volcanic landscapes in Costa Rica and Central America (Pringle & Triska, 2000), inputs of geothermally modified groundwater can potentially result in large spatial heterogeneity within and among streams in this area.

High phosphorus concentrations in geothermally modified streams were also important in explaining patterns in microbial respiration rates. In nine streams, microbial respiration rates on both leaf types (i.e. mixed and *Ficus*) were related to phosphorus, either as SRP or as N : P ratios. In addition, experimental phosphorus enrichment in the Carapa produced an increase in microbial respiration rates on leaves that was of similar magnitude to that measured in naturally solute-rich streams. We consider the change in pH that resulted from phosphoric acid addition to be too small to impact respiration rates. Our results suggest that microbial assemblages in the surveyed streams were responding to differences in phosphorus concentration. We hypothesise that differences in microbial respiration rates among our study streams were, at least in part, the result of differences in microbial biomass. Our standard substrate (i.e. *Ficus* leaves) was incubated for 8 days in each stream before respiration rates were measured. Thus, differences in leaf nitrogen content among streams were probably the result of differences in microbial colonisation during the incubation period (Webster & Benfield, 1986). Respiration rates on *Ficus* leaves were highest on leaves with the highest nitrogen content.

Microbial respiration rates on newly colonised *Ficus* leaves did not respond linearly to the phosphorus concentration gradient. Respiration rates formed an asymptotic curve, increasing to a plateau. Although not significant, the same trend was observed for respiration rates on mixed leaves. Asymptotic curves are characteristic of biotic responses to resource gradients. The Monod and Michaelis–Menten equations have been used extensively to describe nutrient use by microbes (e.g. algae and bacteria); both equations describe a similar response, where microbial growth increases as nutrient availability increases until a saturation point is reached (e.g. Kilham & Hecky, 1988). A similar relationship with phosphorus has been found for other ecosystem processes measured in streams at La Selva. Studies of leaf decomposition along a natural phosphorus gradient (similar to the one studied here) showed an asymptotic relationship between phosphorus and leaf litter decay rates, fungal biomass (as ergosterol) and insect biomass (Rosemond *et al.*, 2002). A previous study at La Selva Biological Station (Rosemond *et al.*, 2002) reported half saturation constants ($K_m$) ranging from 6.5 to 12.8 μg SRP $L^{-1}$ and phosphorus saturation potentially occurring between 25 and 50 μg SRP $L^{-1}$, for the relationships between phosphorus and leaf litter decay rates, fungal biomass, and insect biomass. Our results for microbial respiration rates indicated lower half saturation constants (range 2.8–6.9 μg SRP $L^{-1}$) and examination of the relationship between phosphorus and microbial respiration rates suggested that saturation with phosphorus occurred at concentrations <15 μg SRP $L^{-1}$. The lower values we found suggest that microbial respiration is a more sensitive measure of microbial responses to phosphorus than the decay rates or fungal biomass measured by Rosemond *et al.* (2002).

Responses of microbial respiration to the phosphorus gradient differed between substrate types. While respiration rates on *Ficus* and mixed leaves were related to phosphorus, rates in sediments were not related to any variable measured. The lack of a relationship between sediment respiration rates and phosphorus may have been because of the low quality of sediments as a substrate (e.g. sediments had the lowest nitrogen content of all substrates). Measurement of oxygen consumption might not be the best method to estimate microbial respiration on sediments. Hill *et al.* (2002) found microbial respiration rates on sediments measured as dehydrogenase activity to be higher and more responsive to environmental variables than rates measured as oxygen consumption. Differences in respiration rates between *Ficus* and mixed leaves were probably the result of leaf quality and variable conditioning times. *Ficus* packs were composed of a single species with low C : N ratios, while mixed leaves represented numerous riparian species that differed in their relative quality as carbon sources to microbes. Differences in C : N ratios and coefficients of variation between *Ficus* and mixed leaves were probably the result of differences in stream incubation time. *Ficus* leaves were incubated for 8 days and mixed leaves for an undetermined amount of time. In summary, *Ficus* leaves appeared to be a higher quality and more homogeneous substrate (e.g. lower C : N ratios and coefficients of variation) for microbes than were mixed leaves.

Respiration rates were higher in this study than those reported for most other tropical streams and rivers (Lewis & Weibezahn, 1976; Paaby & Goldman, 1992; Laperrire, 1995), with the exception of those reported for tropical Asian streams (Dudgeon, 1983). The only previous study that measured respiration rates in La Selva streams reported very low rates (<2 g $O_2$ $m^{-2}$ $day^{-1}$) relative to our results. However, these lower measurements were made in small non-recirculating chambers on epilithon attached to artificial inorganic substrates instead of detritus (Paaby & Goldman, 1992). Webster, Wallace & Benfield (1995) summarised data on microbial respiration for temperate streams along the eastern coast of the United States. Their upper range of respiration rates were similar to the phosphorus-poor sites at La Selva, while La Selva phosphorus-rich sites were above the range for temperate streams. The range reported by Webster *et al.* (1995) included summer temperatures of up to 30 °C, which are higher than the ones we measured at La Selva. In a broader-scale study, Sinsabaugh (1997) developed a relationship between stream benthic respiration rates and temperature using data from 22 temperate streams that ranged in temperature from 5 to 19 °C. The relationship predicts that streams at La Selva (with a temperature of 25 °C) will have a respiration rate of 2.4 kg C $m^{-2}$ $year^{-1}$. Although this predicted value is close to mean rates measured in phosphorus-rich streams and to the overall mean, it overestimates respiration rates in phosphorus-poor streams. Overall, high microbial respiration rates at the study streams indicate that microbes are potentially important energy pathways, moving carbon to upper trophic levels, in these lowland stream ecosystems. Inputs of groundwater rich in phosphorus potentially play an important role in increasing the amount of energy flowing through the microbial pathway.

In summary, inputs of geothermally modified groundwater create heterogeneity in stream solute composition. Of all solutes in geothermally modified streams at La Selva, phosphorus was apparently the driver behind variations in microbial respiration rates on benthic substrates (e.g. leaves and sediments). As a result, geothermally modified, phosphorus-rich streams have enhanced microbial activity relative to phosphorus-poor streams. As the presence or absence of upwelling zones of geothermally modified groundwater controls phosphorus levels, landscape geomorphology indirectly influences stream microbial activity.

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