## CAN NUTRIENT SPIRALLING BE USED TO DETECT SEASONAL NUTRIENT UPTAKE IN A FORESTED STREAM?

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Abstract. Nutrient spiralling measurements were conducted in Lyrebird Creek, a forested stream in the Dandenong Ranges, Victoria, Australia. Spiralling indices from several nutrient ( $NH_4^+$ ,  $PO_4^{3-}$ ) enrichment experiments were correlated with seasonal variation in factors thought to control nutrient uptake, i.e., temperature, light and algal biomass. It was hypothesized that nutrient uptake would be higher in summer as increased temperatures would promote both biotic and abiotic processes and higher light levels in summer would stimulate photosynthesis. However, results did not support this hypothesis. Uptake length for  $NH_4^+$ –N and  $PO_4^{3-}$ –P and uptake velocity were not correlated with chlorophyll-*a*, light or temperature ( $r^2 < 0.30$ , P > 0.1) despite the seasonality of these biophysical factors ( $r^2 > 0.42$ , P < 0.02). Lyrebird Creek might had no seasonal trend in nutrient uptake and/or nutrient spiraling measurements only appears suitable for contrasting streams with large differences in biophysical factors that supports biotic and abiotic nutrient processing. In addition, small errors in measuring a nutrient concentration can result in a large range in the estimated  $S_w$  and increased difficulty in determining significant differences in nutrient spiralling indices.

Keywords: nutrient spiralling, forested stream, seasonal variation, biophysical factors, ecosystem processes

## 1. Introduction

Stream processes modify nutrient concentrations during downstream transport (Meyer & Likens, 1979). Thus, an understanding of in-stream processes is vital in managing nutrient movement from the catchment to the receiving waters (e.g., estuaries, wetlands, lakes). Webster and Patten (1979) introduced the concept of 'nutrient spiralling' to explain nutrient movement in lotic ecosystems. As a nutrient atom enters a stream, it is cycled through biotic and abiotic compartments while being transported downstream. The combination of these uptake and longitudinal transport processes, thus resemble a spiral. The distance a nutrient molecule travels while completing a cycle through the biotic and abiotic compartments is called the 'spiralling length' (S) (Newbold, Elwood, O'Neill, & Winkle, 1981) and is commonly measured as the uptake length ( $S_w$ ) (Stream Solute Workshop, 1990). Streams with short spiralling lengths retain more nutrient with less transported downstream.

Spiralling length is measured by injecting a nutrient solution into the stream and observing concentration changes between upstream and downstream sampling stations. To correct for dilution, a conservative tracer is injected simultaneously. Uptake length is then calculated as:

$$S_{\rm w} = \frac{x}{\ln\left(\frac{C_{Nut-Up}}{C_{Nut-Do}}\right) - \ln\left(\frac{C_{Con-Up}}{C_{Con-Do}}\right)} \tag{1}$$

where x is stream reach length,  $C_{\text{Nut-Up}}$  and  $C_{\text{Nut-Do}}$  are nutrient concentrations at upstream and downstream stations, and  $C_{\text{Con-Up}}$  and  $C_{\text{Con-Do}}$  are conservative tracer concentrations at the same stations.

To correct for the effect of discharge, Davis and Minshall (1999) recommended uptake velocity ( $V_{\rm f}$ ) as a measure of benthic demand for nutrients relative to supply.  $V_{\rm f}$  (m/s) is calculated as:

$$V_f(ms^{-1}) = \frac{Q}{w \cdot S_w} \tag{2}$$

where Q=discharge (m<sup>3</sup>/s), w=width (m), and S<sub>w</sub>=uptake length (m).

Spiralling has been used to measure the nutrient utilization capacity of a stream (e.g., Hart, Freeman, McKelvie, Pearse, & Ross, 1991; Kopacek & Blazka, 1994; Mulholland, Newbold, Elwood, & Ferren, 1985), and to compare nutrient uptake between contrasting stream conditions (Maltchik, Molla, Casado, & Montes, 1994; Merseburger, Marti, & Sabater, 2005). There are few studies measuring seasonal variation of nutrient uptake using the nutrient spiralling approach, particularly for the Mediterranean-type climate region in the Southern Hemisphere. We expected higher  $V_{\rm f}$  in summer than in winter as higher temperatures and light intensity in summer should increase nutrient uptake (Butturini & Sabater, 1998; Gardner et al., 2000) through greater algal growth (Hill, Ryon, & Schilling, 1995). Higher temperatures should also increase the kinetics of abiotic reactions (Suplee & Cotner, 2002).

Our purpose was to examine the viability of nutrient spiralling as an indicator of stream function by conducting seasonal nutrient enrichment experiments and investigating correlations of  $S_w$  and  $V_f$  with stream biophysical factors (e.g., algal biomass, temperature, light).

## 2. Materials and Methods

## 2.1. STUDY SITES

Lyrebird Creek drains 724 ha of forested catchment area, 37 km to the east of Melbourne, Australia (37°49.82'S, 145°23.82'E), with only 0.1% of the catchment

covered by hard surfaces such as buildings and roads (Walsh, 2000). The creek is highly shaded by Eucalypts (*Eucalyptus regnans*) with tree ferns (*Cyathea australis*) underneath. Baseflow discharge ranged between 1 and 10 l/s. During baseflow the average stream depth and width were 5 cm and 80 cm, respectively. The stream sediments overlying the bedrock were fine silt and gravel/pebbles covered by organic matter. Large woody debris had accumulated in some sections. Ambient stream water concentrations of  $NH_4^+$ –N,  $PO_4^{3-}$ –P, and  $NO_3^-$ –  $NO_2^-(NO_x)$  were (yearly median at baseflow±%RSD; Taylor, Roberts, Walsh, & Hatt, 2004) 18±8, 3±15 and 310±10, respectively suggesting possible P limitation. Benthic biomass, measured as chlorophyll-*a* concentration (APHA, 2005) varied between 4 and 121 mg/m<sup>2</sup>.

### 2.2. SPIRALLING EXPERIMENTS

Ambient nutrient concentrations were determined from water samples (three to five replicates) collected at upstream and downstream stations prior to nutrient enrichment. Enrichment involved co-injecting  $NH_4^+$  as  $NH_4Cl$ ,  $PO_4^{3-}$  as  $KH_2PO_4$ , and Br as KBr into an 80 m reach, from mid-day until late afternoon, 13 times between December 2002 and March 2004. Nutrient enrichment was kept as low as possible to avoid saturation of benthic uptake (Hart, Freeman, & McKelvie, 1992; Mulholland et al., 2002). Stream water was confined near the injection point (8 m above the upstream sampling point) to ensure complete mixing of the added nutrient solution with stream water. Water samples were collected every 5 min for 90-120 min. Samples were immediately filtered through 0.2 µm GHP membrane filter paper (Gelman-Pall 47 mm dia.), into HDPE tubes, stored on dry ice and kept frozen for later analysis. Flow injection analysis (Lachat Quick Chem 8000) was performed with standard QA procedures (WSC, 2001) using the automated phenate colorimetric method for  $NH_4^+-N$ , molybdenum blue colorimetry for  $PO_4^{3-}-P$ , cadmium reduction method for nitrate-nitrite, and phenol red colorimetry for bromide (APHA, 2005).

## 2.3. BIOPHYSICAL MEASUREMENTS

Stream water temperature and surface light were recorded during each experiment using a calibrated Horiba U-10 meter and Odyssey<sup>TM</sup> light loggers (Dataflow Systems Pty Ltd, Queensland, Australia). Wetted stream width was measured at 5 m intervals after each experiment. Chlorophyll-*a* sampling was undertaken every season. Samples of sediment, rock or large woody debris were taken from 33 random locations along the stream reach, and their surface areas measured. Biomass attached to big rocks or wood was sampled using a 6.6-cm<sup>2</sup> corer. Samples were frozen and stored in the dark before laboratory analysis. The

chlorophyll extraction and determination followed APHA (2005) using a Hitachi U-2000 spectrophotometer.

# 2.4. DATA ANALYSIS

Data was grouped into summer (December–April) and winter (May–July). A general linear model (GLM, Systat Ver. 11) was used to investigate differences in the ambient concentrations of NH<sub>4</sub>–N and PO<sub>4</sub>–P, average chlorophyll-*a* (Chl-*a*), average stream water temperature, and average surface light (light) and  $V_{\rm f}$  and  $S_{\rm w}$ . Light and Chl-*a* data were log<sub>10</sub>-transformed,  $V_{\rm f[PO4-P]}$  was square root-transformed, and  $S_{\rm w[PO4-P]}$  and  $S_{\rm w[NH4-N]}$  was inverse-transformed to minimize the influence of outliers and to normalize the distribution. No transformation was required for NH<sub>4</sub><sup>+</sup>–N, PO<sub>4</sub><sup>3–</sup>–P, and  $V_{\rm f[NH4-N]}$ . Multiple regression analysis was used to examine relationships between spiralling indices and the independent variables (temperature, Chl-*a*, and light).

### 3. Results

## 3.1. BIOPHYSICAL CHARACTERISTICS

Water temperature ranged from 8.5 to 16.9 °C and was significantly different between seasons ( $r^2=0.84$ , P<0.001). As expected, the light climate was significantly higher ( $r^2=0.73$ , P<0.001) in summer (58–579 µmol m<sup>-2</sup>s<sup>-1</sup>) than in winter  $(7-21 \mu mol m^{-2}s^{-1})$ . Biomass responded as expected to seasonal patterns in light and temperature, and was higher in summer  $(1.84 \text{ to } 8.6 \text{ mg m}^{-2})$ than in winter (0.84 to 1.84 mg m<sup>-2</sup>) ( $r^2=0.42$ , P=0.014, n=14) in accordance with Taylor et al. (2004), who previously noted no significant biomass differences within season, but large differences between summer and winter. Stream discharge varied by over an order of magnitude in summer from 1.1 to 44 l/s, and from 6.2 to 21 l/s in winter. The 'high' flows were both on receding limbs of flood hydrographs. Overall, median discharges were lower in summer than winter. Ambient NH<sub>4</sub><sup>+</sup>-N and PO<sub>4</sub><sup>3-</sup>-P concentrations did not vary seasonally ( $r^2 < 0.10$ , P > 0.20; n = 14). In summer, PO<sub>4</sub><sup>3-</sup>-P and PO<sub>4</sub><sup>3-</sup>-P concentrations ranged between (mean  $\pm$  SD, n=5)  $11 \pm 3$  to  $24 \pm 5 \,\mu$ g/l and  $1 \pm 1$  to  $7 \pm 4 \,\mu$ g/l, respectively. In winter, ambient  $NH_4^+$ –N and  $PO_4^3$ –P concentrations ranged from  $8 \pm 4$  to  $23 \pm$ 2  $\mu$ g/l and 4 ± 4 to 7 ± 2  $\mu$ g/l, respectively. These results agree with the ambient nutrient measurements of Hatt, Fletcher, and Walsh (2004) over the same period.

## **3.2. Spiralling Experiment**

The levels of nutrient enriched above the ambient concentration (i.e., nutrient enrichment factors) were 1.6 to 10 times the ambient nutrient concentration.



*Figure 1.* **a** Uptake length ( $S_w$ , in m) and **b** uptake velocity ( $V_f$ , in m/s) in summer and winter. Two negative values for  $S_{w[NH4-N]}$  (*marked with asterisk*) were from experiments conducted seven days after flood events.

Uptake length and  $V_{\rm f}$  for NH<sub>4</sub><sup>+</sup>–N and PO<sub>4</sub><sup>3–</sup>–P did not correlate with the enrichment factors ( $r^2 < 0.09$ , P > 0.2). Figure 1a shows two negative outliers for  $S_{\rm w}$  derived from experiments conducted seven days after winter flood events (28 May 2003 and 30 June 2003).

The  $S_{w[NH4-N]}$  for 30 June 2003 was highly negative, resulting in a very skewed distribution even after transformation. However, another experiment conducted 14 days after a large flood in summer (15 Feb 2005) resulted in the highest  $V_{f[PO4-P]}$  recorded (Figure 1b). For this event,  $S_{w[NH4-N]}$  and  $S_{w[PO4-P]}$  were 1,500 m and 300 m, respectively.

### 3.3. SEASONAL VARIATION AND CORRELATION WITH BIOPHYSICAL FACTORS

The  $V_{\rm f}$  and  $S_{\rm w}$  measurements for NH<sub>4</sub><sup>+</sup>–N and PO<sub>4</sub><sup>3–</sup>–P were not correlated with discharge ( $r^2 < 0.30$ , P > 0.05) and showed no seasonal trends ( $r^2 < 0.30$ , P > 0.05), irrespective of the two outliers, and despite the seasonal differences in the stream's biophysical characteristics. There were no strong associations between these spiralling indices and chl-*a*, light, and temperature ( $r^2 < 0.30$ , P > 0.1). However  $V_{\rm f[PO4-P]}$  showed moderate correlation with light ( $V_{\rm f[PO4-P]}$   $r^2=0.34$ , P=0.03).

#### 4. Discussion

No seasonal pattern was observed in the spiralling indices for  $NH_4^+$ –N and  $PO_4^{3-}$ –P in Lyrebird Creek. This finding may be interpreted in two ways. First, there actually was no seasonal pattern in nutrient uptake. It appears that differences in algal biomass, indicated by Chl-*a* concentrations, between seasons are insufficient to cause detectable differences in the spiralling indices. This suggests that benthic algae are not the dominant factor in nutrient uptake in this stream. Webster and Meyer (1997) argued that organic matter related processes control most of the nutrient uptake in forested streams, and heterotrophs like bacteria and fungi play the dominant role in these processes (Meyer, 1994). The fact that bacterial and fungal biomass are related to the amount and type of organic matter (Findlay et al., 2002) supports the findings that nutrient *S*<sub>w</sub> may correlate with the benthic organic matter (e.g., Mulholland et al., 1985; Webster et al. 2000). In Lyrebird Creek, there was no distinct seasonality in the amounts of coarse particulate organic matter present (subjective observation).

Second, spiralling measurements may not be capable of differentiating seasonal differences due to lack of sensitivity of this technique and/or the inherent variability in stream conditions. Spiralling measures the use of stream nutrients by both the biotic and abiotic components in the stream. Both components are influenced directly and indirectly by nutrients, light and temperature in processing nutrients. The key question is then 'how much change in biophysical factors is required for observable changes in biotic and abiotic nutrient processing?'

Using a single addition technique, Marti and Sabater (1996) found no significant seasonal trend for  $S_{w[NH4-N]}$  and  $S_{w[PO4-P]}$  in a heavily shaded Mediterranean stream. However, after logging of riparian trees, temperature differences between summer and winter were increased resulting in observable seasonal differences for  $S_{w[NH4-N]}$  (but not  $S_{w[PO4-P]}$ ). They found no differences within a season in  $S_w$  at low discharge (<100 l/s) for both nutrients before and after logging, even though the stream detritus input was decreased from 10% surface coverage to 2% (Butturini & Sabater 1998). Simon, Townsend, Biggs, and

Bowden (2005) reported that seasonal patterns in  $S_{\rm w}$  for NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and PO<sub>4</sub><sup>3-</sup> in two riparian grass streams in the southern part of New Zealand were largely influenced by discharge and that there was no seasonal pattern in nutrient  $V_{\rm f}$ .

Using <sup>32</sup>P addition Mulholland et al. (1985) found relatively large differences in  $S_{w[PO4-P]}$  between autumn (shortly after leaf fall), and summer in a forested stream in Tennessee, USA, although no replicate measurements for each season were made. Webster et al. (2000) found differences in  $S_w$  for nitrogen (ammonium and nitrate) and phosphorus after removing all leaf litter and woody debris in one of two similar forested streams (in terms of size and primary production).

These observations suggest that spiralling indices are only sensitive to relatively large differences in biophysical factors such as the observed seasonality in these factors in the northern hemisphere, where most trees lose all of their leaves in autumn adding substantially to the standing stock of organic matter. The riparian Eucalypts have no seasonal leaf abscission, and thus, there are no large seasonal differences in organic matter accumulation. This lack of seasonality of organic matter accumulation is probably the reason we find no detectable seasonal changes in spiralling indices.

A further reason why there is a difficulty in determining significant differences in nutrient spiraling indices is that small errors in measuring nutrient concentrations can result in a large range in the estimated  $S_{w}$ . This is particularly evident in situations where there is low nutrient uptake. This is illustrated using the data for the relatively high and low NH<sub>4</sub><sup>+</sup>-N retention cases of 12 June 2003 and 30 June 2003, respectively (Figure 1b). Assuming a very small error of 2 µg/l in the measurement of the downstream concentrations would result in the  $S_w$  value ranging between 130 and 188 m for the high NH<sub>4</sub><sup>+</sup>-N retention case and between -2,190 and 3,550 m for the low NH<sub>4</sub><sup>+</sup>-N retention case. Larger range in spiraling indices would be expected for low concentration of nutrient (i.e., <20 µg/l) because error in measuring low concentrations is generally higher than the high concentrations (Horwitz, Kamps, & Boyer, 1980).

#### 5. Conclusions

The spiraling technique can only be used for comparison studies when the differences in nutrient uptake between systems are large. Small errors in measuring nutrient concentrations can result in a large range in the estimated spiraling indices, which add further difficulty in determining significant differences in nutrient uptake. These results highlight the need for an investigation into the sensitivity of uptake indices to the magnitude of variation in measuring nutrient concentrations in stream water samples, within the concentration ranges typically used in spiraling experiments. In addition, there is a need to conduct several spiraling experiments in a system that has much larger differences in biophysical factors between seasons.

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