^{13}C and ^{15}N in periphyton in the lower Selwyn River (Canterbury, New Zealand), a groundwater-fed river surrounded by intensive agriculture

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Abstract

Although water quality measurements are widely performed, they do not assess nutrient uptake by stream biota directly. We measured $\delta^{15}N$ and $\delta^{13}C$ in *Spirogyra* to assess analysis of periphyton mats as a means of identifying time-integrated changes in C and N sources. We sampled c.7 km of the lower Selwyn River starting from the river effluence, in April 2003, April 2004, and November 2004. Water quality data were collected in November 2004. Isotopic signatures varied widely, especially for $\delta^{13}C$ (range - 43.6 to - 23.5 ‰). Maximum depletion of $\delta^{13}C$ ($\leq$ -40 ‰) occurred at the river effluence, consistent with significant groundwater C supply at this point. In April 2004, strongly depleted values also occurred near the downstream end of the study reach, implying variable groundwater sources to the river. Periphyton $\delta^{13}C$ and $\delta^{15}N$ were not correlated, indicating decoupled C and N cycling. Periphyton $\delta^{15}N$ downstream of a dairy cattle crossing was significantly higher than upstream values in April 2003, but not in April 2004. Differences in the effect of the cattle crossing on downstream N cycling may be mediated by flood disturbance of excreta-enriched riverbed materials at the crossing. Our results support the use of isotopic analysis of periphyton for identifying nutrient dynamics in the lower Selwyn River.

Keywords: carbon-13 – dairying – eutrophication – nitrogen-15 – nutrients – stable isotopes.

Introduction

In New Zealand and elsewhere, the quality of surface and ground waters is often degraded by intensive agriculture (Hooda *et al.* 2000; Close *et al.* 2001; Hamill & McBride 2003; Houlbrooke *et al.* 2004). The Canterbury region of New Zealand is characterised by a continuing intensification of land use for agriculture (Parkyn & Wilcock 2004) and a complex system of confined and unconfined aquifers (Brown 2001). Intensive agriculture contributes significantly to groundwater contamination, especially with nitrate derived from urine patches and land applications of farm effluent (Close *et al.* 2001; Di & Cameron 2002; Cameron & Di 2004).

The Selwyn River (mean flow 3.4 m^3 / s; Canterbury Regional Council 1997) has a catchment encompassing some of the most intensive agricultural land use in the South Island. In its upper reaches, the river is fed mainly by rainfall runoff and the catchment is less intensively developed. The river then disappears to groundwater beneath alluvial gravels. In its lower reaches, the Selwyn resurfaces as a groundwater-fed river (Brown 2001) surrounded by intensive agricultural activity. The total nitrogen (N) concentration of the surface waters of the lower Selwyn is eight times higher than the upper Selwyn River (Canterbury Regional Council 1997). Because of its hydrology, contamination in the lower Selwyn River could be caused by discharge of polluted groundwater, surface runoff from adjacent farms, and direct access to the river by livestock. Closure of a popular swimming area resulted from direct access to the river and consequent high levels of microbial contamination (Environment Canterbury 2002). Inclusion of river crossings on routes between grazing areas and milking sheds also gives rise to substantial twice-daily inputs to rivers of nutrients and contaminant microbes (Davies-Colley *et al.* 2004); one such crossing occurs in the lower Selwyn River (pers. obs.).

Although water quality measurements of the various forms of N (ammonia, nitrate, nitrite, and organic N) are widely performed, they do not assess N uptake by stream biota directly (Biggs & Kilroy 2004). Because environmental processes often generate characteristic stable isotope signatures, stable isotope analysis has been used to identify elemental sources and processes in a wide range of systems. A dual isotope approach, whereby more than one isotope is determined, can be particularly useful (Handley & Raven 1992). Mineralization of soil organic matter (SOM) typically yields residues enriched in ^{13}C and ^{15}N (Nadelhoffer & Fry 1988; Boutton 1996; Yoneyama 1996) implying depletion of both ^{13}C and ^{15}N in soluble forms leached to groundwater. Consequently, C and N isotopes are obvious partners in studies of soil-water systems. Such a partnership is also convenient, because C and N isotopic analysis is performed simultaneously on the same sub-sample by the same instrument. However, the environmental processes affecting each element differ somewhat, especially in the context of a system potentially affected by intensive agriculture. Oxidation of soil organic matter leads to a $\delta^{13}C$ of soil atmosphere CO_2 that is c. 10-20 ‰ depleted relative to atmospheric CO2 (Palmer *et al.* 2001). Conversely, inputs of animal excreta to soil from intensive agriculture lead to enhanced $\delta^{15}N$ enrichment of SOM, due to ammonia volatilisation (Frank *et al.* 2004; Hawke 2001). Nitrification therefore yields nitrate that is enriched in ^{15}N relative to nitrate from non-agricultural areas, typically +15 ‰ for nitrate from intensive agriculture (Karr *et al.* 2001) compared with -1 to +2 ‰ for nitrate in systems draining forest (Koba *et al.* 1997).

Our study aimed to test natural abundance isotopic analysis of ^{15}N and ^{13}C in periphyton as a means of assessing time-integrated changes in nutrient source along the length of the lower Selwyn. Specifically, input to the river from groundwater enriched in inorganic C from soil organic matter should deplete periphyton $\delta^{13}C$. Leaching from animal excreta from intensive agriculture would be expected to enrich periphyton $\delta^{15}N$, regardless of whether N input is via surface water or groundwater. We were also interested to see if a dairy cattle crossing led to changes in periphyton $\delta^{15}N$. We complemented isotopic analysis with conventional water quality measurements of temperature, conductivity, nitrate, nitrite, ammonia, and organic N. Our study was spread over two years, to see if patterns in isotopic enrichment were consistent.

Methods

We collected samples of periphyton mats at approximately 0.5-1.5 km intervals from where the lower Selwyn River emerges (the effluence) to just below Coes Ford (Figure 1). Sampling intervals were governed by the availability of sufficient material for collection. Although periphyton mats were abundant downstream of the cattle crossing, none was found closer than 1.3 km in the upstream direction. Sampling was carried out on 24 March (three upstream sites) and 3 April (six downstream sites) 2003, 1 April 2004, and 9 November 2004. In May 2003 we sampled one site in the upper Selwyn River not far from the submergence of the river, for comparison with the lower Selwyn data. We measured temperature and conductivity (corrected to 25 °C) at each sampling site, and collected water samples for nitrate-N, nitrite-N, ammonia-N, and organic N analysis (November 2004 only). Two water samples were collected in duplicate to assess possible loss of N containing analytes after collection.

We collected periphyton samples by pulling filaments (c. 5-10 mL drained volume) away from the stones or rocks to which they were attached, pooling from at least four different mats across the river at each site. Samples were thoroughly rinsed in river water to remove particulate material. In the laboratory, the dominant taxon at each site in April 2003 was identified using the pictorial guide of Moore (2000). Samples were dried at 60 °C prior to isotopic analysis. Stable isotope (^{13}C, ^{15}N) analysis was carried out in duplicate using isotope ratio mass spectrometry (IRMS; Europa Geo 20/20) by the Institute of Geological and Nuclear Sciences (Lower Hutt, New Zealand). Results from the isotope ratio analyses were calculated as the per mil (‰) deviation from PDB limestone ($\delta^{13}C$) or air ($\delta^{15}N$) standards.

$$\delta\ (‰) = 1000 \times (R_{Sample} - R_{Standard}) / R_{Standard}$$

R_{Sample} is the ratio of the heavy to the light isotope for the sample, and $R_{Standard}$ is the corresponding ratio for the standard. The 95 % confidence intervals of the differences between the IRMS analysis duplicates were 0.10-0.16 ‰ ($\delta^{13}C$) and 0.08-0.17 ‰ ($\delta^{15}N$).

Water samples for nitrate-N, nitrite-N, ammonia-N and organic N were analysed within 48 hours of sample collection by Chemsearch Ltd (University of Otago, Dunedin), after storage in the dark in an insulated bag or box during field sampling and courier transport. Colorimetric analysis for nitrite and cadmium-reduced nitrate used diazotisation with N-(1-naphthyl)-ethylenediamine; ammonia was measured using the colorimetric phenate method; and organic N was analysed by Kjeldahl digestion. Detection limits were 10 mg / m^3 (organic N), 1 mg / m^3 (ammonia-N), and 5 mg / m^3 (nitrate / nitrite-N).

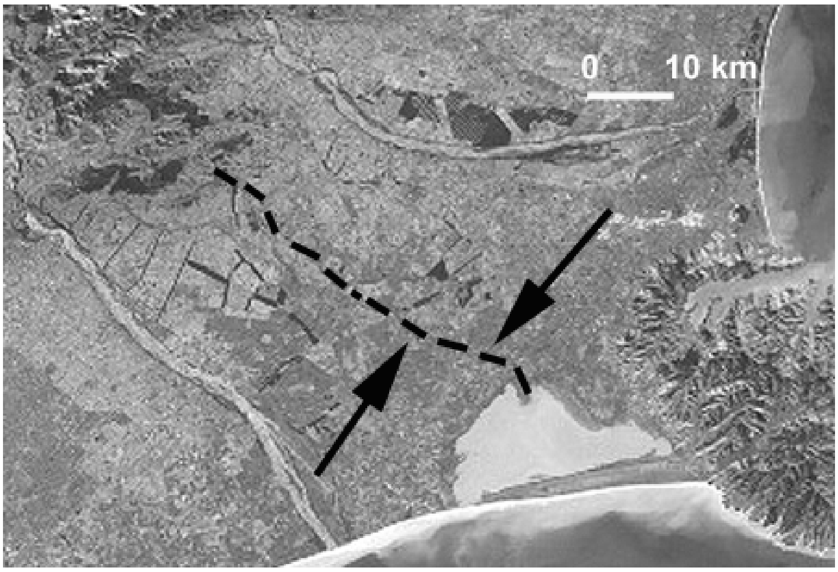

Figure 1. Photograph of the path of the Selwyn riverbed (dashed line) across the Canterbury Plains, in the interfan depression between the Waimakariri River (top) and the Rakaia River (bottom), to Te Waihora/Lake Ellesmere. As noted in the text, the riverbed is normally dry across much of the Plains. The section of river sampled was between the two arrows. (Image courtesy of NASA Earth Observatory.)

Sampling duplicates for dissolved N species agreed either within 0.1 g / m^3 (nitrate-N), or exactly (remaining analytes), thus validating the sampling and analysis protocols.

Where possible, statistical analysis of the data used non-parametric methods to avoid assuming particular statistical distributions. Spearman's Rank was used to assess correlation, while the Mann-Whitney *U*-test was used to compare different populations. In situations where small sample sizes invalidated use of the Mann-Whitney *U*-test, a Welch-corrected *t*-test was used.

Results

The range of isotopic enrichments was -43.6 to -23.5 ‰ ($\delta^{13}C$) and -0.2 to +7.0 ‰ ($\delta^{15}N$). Values for both isotopes fluctuated between years at particular sites (Figure 2). Overall, the $\delta^{13}C$ data sets were not significantly different (Mann-Whitney $U = 48.0$, $P = 0.65$), but $\delta^{15}N$ data for 2003 were significantly higher than for 2004 (Mann-Whitney $U = 17.0$, $P = 0.006$). Periphyton $\delta^{15}N$ and $\delta^{13}C$ were not significantly correlated in either April 2003 (Spearman $r = 0.21$, $P = 0.54$) or April 2004 (Spearman $r < 0.0001$, $P > 0.999$). Sample size was insufficient for a correlation analysis of the November 2004 data. *Spirogyra* was the dominant (> 90%) periphyton taxon in all April 2003 samples, and was assumed for subsequent samples.

Periphyton mats were found only over the upper 2.9 km of the river in November 2004, probably reflecting re-growth after a flood 2 months earlier (see Discussion). The river emerged at different places on the 3 sampling occasions, consistent with different mean

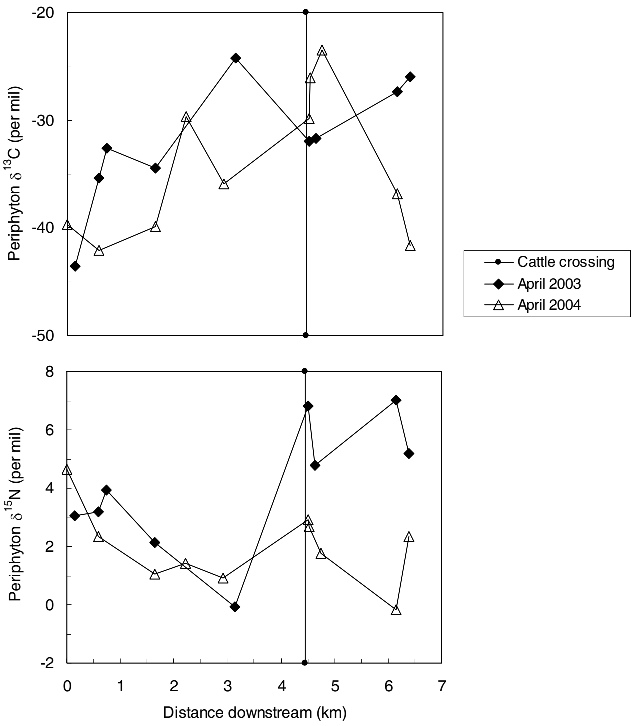

Figure 2. a) $\delta^{13}C$ and b) $\delta^{15}N$ values for periphyton from the lower Selwyn River in April 2003 and April 2004, in relation to a dairy cattle crossing. The furthest upstream data for both samplings were collected from the immediate vicinity of the river effluence. The zero distance on the *x*-axis represents the effluence point in April 2004. River flow data mentioned in the text were collected from Coes Ford, at 6.3 km.

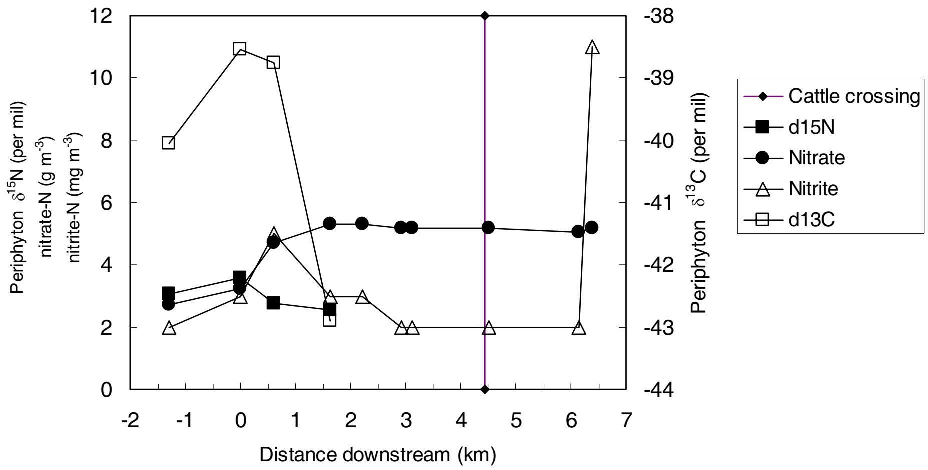

Figure 3. Periphyton $\delta^{13}C$ and $\delta^{15}N$ and dissolved nitrate-N in river water from the region of the lower Selwyn River effluence, November 2004. The zero distance on the *x*-axis represents the effluence point in April 2004.

daily flows (measured at Coes Ford, near the downstream limit of sampling). River emergence was 6.3 km upstream from Coes Ford in April 2003 (mean daily flow 0.185 m^3 / s), then 145 m further upstream in April 2004 (0.258 m^3 / s) and 1.29 km further upstream in November 2004 (1.025 m^3 / s).

Periphyton $\delta^{13}C$ was significantly correlated with distance downstream in April 2003 (Spearman r = 0.817, P = 0.011) but not in April 2004 (Spearman r = 0.297, P = 0.41). In contrast, periphyton $\delta^{15}N$ was not correlated with distance downstream on any occasion (April 2003, Spearman r = 0.650, P = 0.066; April 2004, Spearman r = -0.249; P = 0.49).

On all three sampling dates, periphyton $\delta^{13}C$ showed extreme depletion (≤ -40 ‰) close to the point at which the river emerged from the ground (Figures 2 & 3). Downstream values were much higher, with the highest approaching the single upper Selwyn value (-23.3 ‰). Considerable differences were found between sampling dates in the values of $\delta^{13}C$ close to the effluence. In April 2003, extreme depletion of ^{13}C was observed only in the immediate vicinity of the point of effluence. In contrast, on other sampling dates depletion was observed for ≥1.6 km downstream. In November 2004, depletion increased sharply c. 2.5 km downstream of the effluence (Figure 3), at a point close to where the region of extreme depletion ended in April 2004 (Figure 2). In April 2004, extreme depletion of ^{13}C was also observed in the lower reaches of river, c. 6 km downstream from the effluence; no corresponding depletion was found in April 2003.

Patterns in periphyton $\delta^{15}N$ differed between sampling dates (Figure 2). In April 2003, $\delta^{15}N$ increased by 6.7 ‰ immediately below a crossing used by dairy cattle moving between grazing areas and a milking shed. The mean $\delta^{15}N$ upstream of the cattle crossing was significantly lower than downstream (P = 0.008, Welch corrected $t_{0.05, 6}$ = 3.93; 95 % confidence interval of difference, 1.3-5.7 ‰). The values upstream of the cattle crossing encompassed the single upper Selwyn periphyton $\delta^{15}N$ (+3.1 ‰). However, in April 2004 $\delta^{15}N$ values for algae upstream and downstream of the crossing were not significantly different (P = 0.85, Welch corrected $t_{0.05, 7}$ = 0.19).

Water quality data were obtained only in November 2004. Conductivity increased only slightly from 195 to 214 μS_{25} / cm at the cattle crossing. At the upstream end of the study reach, conductivity increased sharply from 162 μS_{25} / cm at the effluence point to 200 μS_{25} / cm over the same 3 km distance as the nitrate increase, and then remained approximately constant at 189-195 μS_{25} / cm. Temperature increased only slightly downstream as the river was sampled, from 15.3 to 18.5 °C, over the 7.7 km of river sampled. Organic N and nearly all ammonia concentrations were less than the detection limit. Nitrate concentrations doubled from the effluence to c. 3 km downstream. Thereafter, concentrations remained constant at 5.1-5.2 g N / m^3 (Figure 3, including data at 3.1, 4.5, 6.1 and 6.4 km), including downstream of the cattle crossing. Nitrite-N only exceeded 3 mg / m^3 at two locations; 1.9 km downstream of the effluence (5 mg / m^3), and below Coes Ford where it increased five-fold from 2-11 mg N m^{-3}. Downstream of the cattle crossing was the only location where ammonia-N concentration reached the lower detection limit. Both $\delta^{13}C$ and δ ^{15}N of periphyton reached their minima about where nitrate reached its maximum (Figure 3). Periphyton mats were absent downstream of this point.

Discussion

At -43.6 to -23.5 ‰, the range of our periphyton δ ^{13}C data was much wider than for in-stream primary producers reported from most other studies. The range is especially large given that the data were collected over a comparatively short distance within a single stream. By way of comparison, Collier *et al.* (2002) reported a range of -23 to -22 ‰ for algae from two streams draining native forest and forest/agricultural catchments. Hollows *et al.* (2002) reported a range of -25 to -33 ‰ for moss from three unshaded streams from pasture catchments, but 95 % confidence intervals within each stream were << ± 1 ‰. Palmer *et al.* (2001) found that δ ^{13}C in aquatic plants was -30.5 to -33.5 ‰ in a stream dominated by terrestrial sources of C. Because our samples were dominated by one taxon (*Spirogyra*), the large range was not due to downstream changes in the periphyton community (Winterbourn *et al.* 1986).

We consider that the wide periphyton δ ^{13}C range we observed to be a reflection of the complex groundwater sources feeding the Selwyn River (Anderson 1994; Brown 2001). Data from the lower Selwyn effluence were similar to the -45 to -46 ‰ reported by Rounick & James (1984) for aquatic plants from a sub-alpine spring. Our periphyton δ ^{13}C results therefore support the hypothesis that periphyton δ ^{13}C reflects uptake of different sources of inorganic C, groundwater supplying significant C (depleted in $^{13}CO_2$) for periphyton near the effluence in the weeks or months prior to all sampling dates. Disappearance of the depletion signal over relatively short distances downstream implied rapid exchange of groundwater-derived CO_2 with the atmosphere.

Our second hypothesis related to the enrichment of periphyton δ ^{15}N arising from intensive agriculture. Supporting this hypothesis, our δ ^{15}N data were substantially more enriched than the -2 ‰ reported for algae from a forest stream and -8 ‰ from an agricultural stream in the North Island (Collier *et al.* 2002). Similarly, Jones *et al.* (2004) found that δ ^{15}N of epilithon and macrophytes gave a useful indicator of nutrient limitation in lakes. However, interpreting the lack of a clear pattern in δ ^{15}N in the region of groundwater discharge (identified by periphyton δ ^{13}C) is difficult in the absence of data on the δ ^{15}N signature of various sources of N reaching the river. If groundwater N was derived from mineralization of SOM largely unaffected by animal input, then a depleted δ ^{15}N would be expected (Yoneyama 1996). In such an event, the relatively enriched periphyton δ ^{15}N found at the effluence would indicate that sources other than groundwater dominated N supply. Considering the intensive land use of the areas surrounding the river, agricultural run-off would be the most likely source. Conversely, if groundwater nitrate was derived predominantly from urine patches (Di & Cameron 2002), then an enriched δ ^{15}N would be expected (Karr *et al.* 2001). N from groundwater, rather than agricultural run-off, would then be the more important in the lower Selwyn. At present, however, no definite conclusions can be drawn.

The effect of the cattle crossing on

periphyton $\delta^{15}N$ differed between years. Results were consistent with a major impact prior to the April 2003 sampling, but not the sampling in April 2004. Enhanced periphyton $\delta^{15}N$ downstream of a cattle crossing reflects ammonia volatilisation from excreta at the cattle crossing, enriching the residual material (Frank *et al.* 2004; Hawke 2001; Karr *et al.* 2001). However, in 2004, there was no effect on either periphyton $\delta^{15}N$ (April data) or nitrate concentration (November water quality data), although ammonia and nitrite concentrations and conductivity all increased. The differences may relate to the morphology of the crossing. Under low flow conditions, the river channel is relatively narrow and deep, and is confined to the true left of the riverbed. The remainder of the riverbed crossed by the cattle consisted of up to 10-20 cm stones and boulders encased in a matrix of cattle dung. Flood events are likely to flush out this nutrient-rich matrix, which could then be dispersed downstream as a particulate plume. Some of the particulates are likely to be trapped, and release their nutrient load over time (or until a major flood flushes them out, as may have happened in November 2003). A lack of flooding at crucial times during the periphyton growing season in spring and summer 2003-4 may have led to a less significant supply of nutrients downstream, and hence no cattle crossing effect for periphyton $\delta^{15}N$ in April 2004.

Traditional water quality measurements indicate the potential for augmenting plant growth without identifying uptake processes. Results of our study supported isotopic analysis of periphyton mats as a means of determining time-integrated nutrient availability in the Selwyn River, so that isotopic analysis of periphyton is complementary to traditional methods. Determining the length of the integration time for isotopic analysis requires knowledge of periphyton mat lifetime, which is primarily determined by flooding events (Biggs 1995; Biggs & Close 1989). Superimposed on the effects of floods is a seasonal growth peak in autumn previously noted from the lower Selwyn (Winterbourn 1974), seasonal patterns often being observed in groundwater-fed streams (Biggs & Kilroy 2004). Flood events, defined as 2.5 times a base flow of 1 m^3 / s, were assessed using mean daily flows at Coes Ford. These showed that the April 2003 data were collected 9 months after a 2.9 m^3 / s flood, the April 2004 data 5 ½ months after a major 17.6 m^3 / s flood, and the November 2004 data 64 days after a 2.8 m^3 / s flood. However, the individual periphyton cells sampled in April 2003 and April 2004 data are unlikely to have been continuously and consistently growing since the flooding date many months earlier. Accurate assessment of the nutrient uptake integration time in the lower Selwyn River will therefore require close observation of periphyton accrual dynamics in concert with isotopic analysis.

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