Ref 3: Review of Bernal et al. Riparian and in-stream controls.

This is a pioneering and potentially an important contribution to the long standing question of whether, how, and to what extent in-stream biogeochemical processes influence stream chemistry. The question has sparked debate over whether in-stream processes alter chemistry enough to compromise the interpretation of stream exports as a measure of terrestrial watershed losses, and therefore whether inferences based on the small watershed concept need to be reassessed. It has been suggested that if in-stream processes are important enough to make a difference, then this should be revealed by longitudinal concentration gradients reflecting the removal or addition of nutrients to the water column. And, in fact, a number of studies have linked net uptake to declining downstream concentrations. But because nutrients enter (and leave) the stream laterally along it length, the notion a net uptake necessarily generates a declining concentration gradient is a simplistic fallacy. (See Eq. 4 of Brookshire et al. 2009 Ecol. 90:293, which is correct but misinterpreted by those same authors). The work under review demonstrates (1) that in-stream processes may strongly affect stream chemistry and yet leave concentrations longitudinally uniform (or otherwise varying), as did Meyer and Likens (1979 Ecol. 60:1255), and (2) the magnitude of biotic effects can be inferred using a mass balance approach that fully accounts for lateral inputs and losses. The authors estimated the effect on stream chemistry of uptake or release by taking the difference between inputs and outputs assessed on each of 15 segments of a 3700 m reach of reach. The assessments were synoptic, repeated 11 times over a year and a half. No one has done this before, at least on this scale. The approach has limitations. We do not get an annual budget and a "sink" is simply the instantaneous difference of inputs and outputs: We do not see whether the nutrient is accumulating, being transformed, or being lost to the atmosphere. However, we do see detailed spatial resolution, revealing a surprising degree of spatial heterogeneity and, wherever there are lateral losses as well as gains, mass balance even at the whole-reach scale, cannot be assessed without it.

I use the caveat "potentially" because I think the presentation is weak in several ways and falls far short of what this amazing data set could support. The major problem, I think, is that the authors are trapped by the misconception that in-stream processes should produce longitudinal gradients. They often do, but they don't necessarily and there are good reasons why they wouldn't. I think this could be a very powerful paper if directly challenged the misconception, and showed how streams can strongly affect concentrations without generating longitudinal gradients. Much more on this below. I have trouble understanding the basic message or messages of the paper, find that important information is missing, see a number of inconsistencies in presentation, and suspect that there may be some major errors. I hope all these can be resolved because this is important work.

First, here is what I consider missing:

(1) A whole reach budget, or budgets (by period, or sampling date). The whole-reach budget summarizes the big picture and will, in the long-run, be an essential, citable result of the paper. Moreover, what happens segment by segment must add up to the whole reach, so it provides closure. A budget would consist of upstream inputs, tributary inputs, groundwater inputs, groundwater losses, in-

stream source-or-sink, and downstream export, i.e., the same as your segment (Eq. 2) budgets, for water, chloride, and nutrients, reported in mass/time (not area-specific).

(2) The map shows many unmeasured tributaries that account for roughly half as much contributing area as the measured tributaries (a table of sub-basin areas would be helpful). The way inputs from the unmeasured tributaries were incorporated into the mass balance should be described. If they were dry, this should be so stated.

(3) The seasonality of rainfall and flow should be provided. Knowing that dormant and vegetative season flows were similar is not sufficient because we would expect increasing baseflows throughout the dormant season and declining flows throughout the vegetative season.

(4) We need more information on groundwater gains and losses, especially the gross inputs and losses over the reach. The assessment of groundwater contribution requires both inputs and losses, not simply a net inflow. .Also needed is an explanation of the groundwater input shown in Fig 3. c. If it is cumulative with watershed area, as is Qspf in Fig. 3a, then Fig. 3 c tells us that, over the whole reach, there was no net gain from groundwater, yet the longitudinal increase in flow (L/s, not area-specific) was substantially greater than explained by measured tributary inputs.

(5) We need a more complete presentation of the Fsw data, longitudinal and temporal. These are the real contribution of the paper, but we see only the whole-study averages, standard errors, and a graph of the frequency of positive and negative estimates.

(6) We need an acknowledgment of the human activity in the lower part of the catchment. I see buildings and agricultural or horticultural activity on Google Earth. As much as 15 hectares of riparian area may be disturbed. This much activity could account for the higher N and P concentrations in the lower-most segments. If you are sure that the activity is inconsequential, you should explain this.

(7) Fig. 5 shows no tributary data for the vegetative season. Tributaries were the major fluvial input, so these data are critical to understanding what was influencing concentrations.

(8) Correct units for |Fsw/Fin|. These would be inverse meters (like a longitudinal loss rate) except that the values are so high that I assume that Fsw is actually Fsw*x.

The message:. I see two contradictory messages emerging from this paper. One is that in-stream biogeochemical processes can be evaluated from mass balance considerations and are large. The other is that the signal from these processes is overwhelmed by other factors—inputs, losses, riparian processes, and landscape features—so that the stream chemistry doesn't reveal what the biogeochemical processes are doing. Both can't be true. Your equation (2)-the statement of mass balance—says that the stream chemistry does reveal biogeochemical processes. It does not make sense to say that "other factors…may overcome the effect of…processing" (598:6), or that "longitudinal trends…were not consistent with…biogeochemical processes" (598:19, 618:25), or that "sources…can offset the effect of nutrient cycling on stream water chemistry" (619:5). If an in-stream process removes some nutrient then it unequivocally affects stream concentration, and the effect can be measured (the point of Eq. 2). It is not overcome, made inconsistent, or offset by other factors. The problem is the implicit equating of an effect on concentration with the presence of a longitudinal gradient. This shows up most clearly in the first sentence of the abstract and as a "hypothesis" in the discussion (614:22). More careful wording would resolve the problem: Clearly state that you are not talking about an effect on concentration but about the expectation that an effect shows up as a longitudinal gradient. Your work shows that a longitudinal gradient does not equate to an effect on concentration. It also shows that biogeochemical processes have a strong effect on concentration—that require careful mass balance to see.

One way to clarify the message might be to focus on how in-stream processes can be large without creating a concentration gradient. The basis for this lies in Brookshire et al's (2009) equation 4: Biological uptake draws the concentration groundwater that enters the stream laterally (presuming that it is higher than the streamwater concentration) down to the concentration of the streamwater that entered the reach. Thus the streamwater concentration shows no gradient, but is maintained lower than the concentration of its sources. Below I question whether your reported in-stream processes (Fsw) are too high, but regardless, it would be instructive to drill down on the high values, asking whether these were associated with segment-specific concentration declines or high groundwater inputs.

The confusion in message extends to the treatment of riparian and landscape factors as variables that explain stream concentrations. The glm model leaves out Fsw despite your finding that in-stream processes affect ammonium and phosphate fluxes by 29 and 30% in a single segment. Instead it includes D (the gradient) making the implicit assumption that in-stream processes can only be represented by a gradient. The image I get is that of two demons—in-stream processes and riparian characteristics—competing for control of stream concentrations. But you already know how much each is controlling, from the mass balance calculations, and don't need statistics for that. The riparian and landscape variables can affect concentrations either through affecting inputs (tributary and groundwater fluxes) or by affecting in-stream processes (e.g., wider stream increases uptake per unit distance, or opens the canopy to primary production). Because the inputs have been measured, the analysis should separate these pathways: one analysis for riparian concentrations (or inputs) and one for in-stream processing (Fsw). For example, terrestrial nitrogen-fixation should be related to its effects on riparian concentrations.

In-stream process estimates: The Fsw and |Fsw/Fsin| values for ammonium and phosphate are surprisingly large (except, perhaps for nitrate), so large that I suspect an error. What follows is an explanation of why I suspect an error. I may be wrong, but either way, I think the paper should provide the data and considerations needed to answer the questions I raise here—because other readers would surely raise them as well. Fsw for ammonium is reported to average 0.6 ± 0.2 (SE) ug/m/s which, for the 3700-m reach is a net uptake of 2220 ug/s. The inputs to the reach from upstream are roughly 200 ug/s and the downstream export is about 800 ug/s, for a net export of 600 ug/s. Thus an input of 2800 ug/s from lateral (tributary and groundwater) sources is needed to balance the budget. The net inflow of water from these sources is about 70 L/s, so the average concentration of the lateral sources would have to be about 40 ug/L. This is far higher than the reported concentrations for both tributary and groundwater, especially tributary which supplies most of the water. The needed concentration may be lower than 40 ug/L if there is a large exchange of groundwater but this exchange would have to be quite large. Thus I suspect that the Fsw of 0.6 ug/m/s is erroneously high. Based on the data available in the paper I calculate that the average Fsw could be no higher than 0.2 ug/m/s, even allowing for substantial additional gains through groundwater exchange. (This conclusion could change if vegetative-season tributary inputs, missing from Fig. 5, were much higher than in the dormant season). Also, the mean Fsw of 0.6 ug/m/s translates to an areal net uptake (U) of about 0.27 ug/m2/s, which seems implausibly high, at least for a mean. It is far greater than the median of 0.03 ug/m2/s reported by von Shiller et al. 2011. And it is about half the gross uptake typical of second and third order reaches (Ensign and Doyle 2006 JGR 111:G04009), which we expect to be much higher than net uptake because retention in stream biota occurs on a timescale of days or weeks.

My concerns are similar for phosphorus, for which the mean Fsw = 0.2±0.4 ug/m/s. In this case the large SE indicates that the net uptake is not statistically significant, yet a net uptake is claimed (598:16). Using the whole reach mass balance approach that I used for ammonia above, I find that the average FSW of 0.2 ug/m/s is reasonable only for the dormant period, and only if the high downstream concentrations are ignored (in which case P concentrations are longitudinally uniform). If the downstream concentrations are included, then I estimate FSW at -0.20, i.e., a net P release. This matches your lower error bound, but because this is simple arithmetic and not statistical sampling, your average should be correct. The large apparent release of P comes from the lower 3-4 stations. If we consider only the reach upstream of 3000 m, then I estimate a net uptake of about 0.1 ug/m/s. I did not check the vegetative period, but unless the tributary concentrations were much higher than in the dormant period, there is no way that there could be a significant net uptake in the vegetative period, over the whole reach or even the upper 3000 m. For nitrate, I'd say that your negative Fsw is entirely driven by the high downstream concentrations in the vegetative period. I get a net uptake for the dormant period, and a net uptake for both periods in the upper 3000 m.

One source of error may be in the measurements. The mass balance calculations depend on accurate measures of both concentration and flow and depend on small differences in sometimes small numbers. The errors are made worse by multiplying these estimates together. Some evaluation of the role such errors should be included.

Error may also come from averaging Fsw (assuming you have divided by x). The segments are unequally weighted with the fourth segment contributing only one-quarter as much as it should.

Groundwater and the riparian interface: The conceptual framework is not entirely clear. The use of mass balance requires a boundary within which processes are measured (as Fsw). For clarity I would suggest the following: The boundary is the riparian-stream edge, the stream channel is inside, and the riparian zone is outside. Fluxes pass through the stream edge, but no processes can occur "at the stream edge" (cf.617:3), only on one side or the other. In re-reading, I see that your suggestion that the apparent downstream nitrate source represents nitrification of riparian ammonium that occurs "at the riparian-stream edge" (616:14). From the standpoint of the Eq. 2, this process occurs inside the boundary, i.e., in the stream channel, the nitrogen having crossed the boundary as ammonium. So you could define "stream-edge" this way, clarifying that it is inside the riparian-stream boundary. If this nitrification actually explains the downstream increase in nitrate concentration, you should be able to demonstrate that Fgw of ammonium was sufficient to account for the negative Fsw of nitrate in those segments.

As you formulated the mass balance, all groundwater fluxes to and from the stream pass through the riparian zone. If hillslope groundwater is to explain phosphorus concentrations in the stream (616:1-20), it must pass through the riparian and would have been measured there. The possibility that hillslope water flows bypasses the riparian, e.g., via underflow, to reach the stream is not consistent with Eq. 2. That is, to invoke hillslope groundwater you must acknowledge that the mass balance is incorrect. Regardless of how you handle the hillslope question, you should explicitly address the question of whether the riparian well samples correctly characterized the groundwater that reached the stream.

Is there an explanation as to why the chloride differed between periods even though the flow did not? At 614:13 you suggest that evapotranspiration (ET) may explain the higher chloride in the vegetative-period, which makes sense except that the greater ET should also have reduced flow. Higher precipitation during the vegetative season, if it occurs, could compensate for the additional ET, explaining the similar flows.

The 40% longitudinal increase in chloride seems higher than can be explained by the decrease in area-specific discharge and hence by ET. In support of the ET suggestion you state that chloride was higher in the main stream than in the tributaries (614:11). But Fig. 3b shows the opposite: Tributary chloride was higher than the mainstream and this, rather than ET, appears to explain much of the increase.

Temporal variation: The absence of a temporal perspective (beyond the dormant/vegetative classification) is surprising. Most streams exhibit distinct seasonal variations in both flow and nutrient chemistry, and these patterns have proven critical to understanding biotic influences on stream chemistry (e.g., Mulholland et al.'s many papers on Walker Branch). If neither precipitation nor flow vary with season, this should be clearly stated. Otherwise we should at least be informed of the seasonal patterns and, ideally, your analysis would make use of them. You do state that dormant and vegetative flows were similar. However, if precipitation is uniform we would expect flow to reach a minimum at the end of the vegetative period and a maximum at the end of the dormant period, with the result that the average of the two seasons is the same.

Page:line-referenced comments:

610:22. "According to the glm results, this decrease was positively related to basal area…" etc. These are the opposite of what is given in Table 3, which shows how these factors would relate to an increase, not a decrease. According to Table 3, basal area increases nitrate, but % N-fixers decreases it. In the GLM the independent variables clearly suffer from collinearity, as they all vary consistently with downstream direction. Collinearity leads to erratic regression coefficents, which may explain some of the seemingly odd relationships.

614:17. "…suggesting…a gaining reach"? You should know from your flow measurements whether it was or was not gaining.

614:23." We found a decreasing longitudinal pattern of stream NO_3^- concentrations, though only during the dormant period." Fig. 5 clearly shows that nitrate decreased in both seasons above 3000 m. Don't let the regression model obscure reality.

614:27-615:2 I disagree. It makes little sense to ignore the clear change in nitrate gradient that occurred at 3000 m, in both periods. It looks to me like in-stream processes were important for the downstream decline above 3000 m. I would say |Fsw/Fin|, at 11% (per segment, right?), is large. Positive values were much more frequent than negative values in the 11 segments above 3000 m. Tributary concentrations were low, so also contributed to the decline. You should calculate how much was in-stream and how much was dilution.

615: 18 The lack of correlation with N2-fixing species does not rule out their possible influence because there was an opposite nitrate gradient in the region upstream of 3000 that would have obscured the correlation in the lower reaches.

615: 22 Release of nitrate from the streambed raises the question of the ultimate source of the nitrogen. Simple storage and release cannot produce a net release; the high concentrations at one time would have to be balanced by low concentrations at another. On 616:14-18 your suggest that the source may be nitrified ammonium. Perhaps that should be mentioned here. Another possibility is in-stream N fixation. See Finley et al. 2011 Ecol. 92:140 who saw a sharp downstream increase due to N-fixation.. Also, as mentioned above, I am concerned about human sources.

616:1 Stream fluxes did not decrease downstream; what decreased was area-specific flux. The absolute nutrient fluxes increased greatly.

616:3 "…concentrations increased from the top to the bottom of the reach for all nutrients (except for NO3 during the dormant period." Nitrate did not increase in the vegetative period either, and ammonium did not increase in the vegetative period.

617:13 "especially during the vegetative period" should read "although only in the vegetative period".

617:25-28. This sentence does not quite make sense. The first part refers to "predominant" in-stream processes while the second part effectively states that there was not a net uptake or release along the reach, i.e., that there was no "predominant" process. As I have argued, it is fallacious to say that a longitudinal trend is consistent or in-consistent with in-stream processing, as it is a matter of balance with lateral inputs. I think that for nitrate above 3000 m, you had both net uptake and a declining trend although low tributary concentrations may also have contributed to the decline. For ammonium and phosphate above 3000 m you had net uptake and no decline because the uptake drew down the concentrations of lateral inputs, i.e., uptake balanced lateral inputs. In my view this is a common case in streams where there is a net uptake. However, this is hard to document from the literature, Meyer and Likens (1979) and Alexander et al. (2009 Biogeochemistry 93:91) being the only two examples I can readily find. This is why your study is so important.

618:14 delete “through” (an apparent typo) and “profound” (exaggeration).

619:19 “can offset the effect of” should read “must be taken into account when interpreting longitudinal gradients.”