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Forest stream biota carbon : nitrogen ratios are unaffected by nutrient subsidies from breeding Westland petrels (*Procellaria westlandica*)

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Abstract. Stable isotope studies have repeatedly shown marine nutrient incorporation from seabirds, anadromous fish and tidal wrack into terrestrial and freshwater ecosystems. However, little is known about the physiological consequences of marine-derived nutrient subsidies. Protein content and lipid storage are important physiologically, and the C : N ratio is a widely used proxy that reflects changes in these quantities. In this study we tested the response of C : N ratios in stream biota to the presence of marine-derived nutrients from Westland petrels, a forest breeding seabird. Samples of different stream invertebrate functional feeding groups, predatory freshwater fish, coarse particulate organic matter and riparian soil and vegetation came from four reference streams and four streams with a wide range of seabird densities. Samples were analysed for percentage C, percentage N and $\delta^{15}N$ using isotope ratio mass spectrometry. The data were tested against petrel colony size, stream size and distance from colony to sampling site. Despite increased $\delta^{15}N$ accompanying petrel presence (as reported previously), Westland petrels had no effect on stream biota C : N ratios, regardless of colony presence or absence or colony size. Despite the nutrients provided by petrels, we conclude that petrel N replaced rather than enhanced non-marine N in these stream ecosystems.

Additional keywords: allochthonous, ecological stoichiometry, marine-derived nitrogen, seabird, stable isotope.

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Introduction

Beginning with the insights of oceanographer Arthur C. Redfield in the 1930s, elemental ratios (most often carbon : nitrogen, C : N; carbon : phosphorus, C : P; or nitrogen : phosphorus, N : P) are an easy-to-measure and conceptually powerful way of establishing the pathways taken by chemical elements through an ecosystem (Sardans *et al.* 2012). Expressed as ecological stoichiometry, these stoichiometric relationships also reflect resource quality (Hessen *et al.* 2013; Frainer *et al.* 2016). Resource quality, whether measured by stoichiometric relationships or by the presence of particular key biomolecules (Twining *et al.* 2016; Sperfeld *et al.* 2017), profoundly affects the growth and reproduction of animals from birds (Fairhurst *et al.* 2015) to invertebrates (Kerpel *et al.* 2006), thereby affecting ecosystem structure and composition.

Freshwater ecosystems can be N limited, P limited or colimited, and the stoichiometry and biomass of primary producers at the base of the food web often responds to nutrient enhancement (Elser *et al.* 2007; Trochine *et al.* 2017). However, colimitation often predominates, with alternation between different limiting elements depending on the spatial and temporal scale (Sperfeld *et al.* 2012). As with many ecosystems, the nutrient status of streams can be affected by natural or anthropogenic cross-boundary transfers, which can occur at both local and landscape scales (Polis *et al.* 2004; Sudduth *et al.* 2013; Sitters *et al.* 2015). Anadromous fish and seabird breeding both function as landscape-scale ecosystem engineers (Smith *et al.* 2011). Colonial seabirds enrich the soils and plants of their breeding and roosting sites, whereas anadromous fish returning to spawn provide direct inputs to streams (Janetski *et al.* 2009;

Mulder *et al.* 2011). Secondary processes transfer the fish-derived nutrients into riparian biota (Naiman *et al.* 2002; Hicks *et al.* 2005) and seabird-derived nutrients into streams (Harding *et al.* 2004).

Seabird inputs to soil are dominated by guano, and its input dominates ecosystem nutrient fluxes to the extent of overwhelming the nutrient binding capacity of soil (Furness 1991; Hawke and Newman 2004). Seabird guano typically has a diet-dependent P-rich N : P stoichiometry relative to recipient ecosystems (Burger *et al.* 1978). In the case of Westland petrels (*Procellaria westlandica*), guano N : P stoichiometry ranges between 1.9 : 1 and 7.4 : 1 by mass depending on the time of year (Hawke 2005), compared with a foliar N : P threshold for N limitation of ~12.5 : 1 (Tessier and Raynal 2003; Richardson *et al.* 2004).

Mediation of seabird inputs by soil may further decrease the N : P stoichiometry of material reaching streams. This stoichiometric change can occur through N loss to the atmosphere, P contributions from soil parent material and solubilisation of refractory P in seabird soil (Hawke 2005; Hawke and Condron 2014). However, these processes depend considerably on soil properties. Ammonia volatilisation and denitrification are governed by soil cation exchange capacity, pH, moisture and texture (Whitehead and Raistrick 1993; Balaine *et al.* 2016), and soil parent material varies widely in its P content (Walker and Adams 1958). Nevertheless, the combination of a P-rich guano stoichiometry, the post-deposition losses of guano N and the solubilisation of soil P suggest that streams affected by seabird inputs will be forced towards N rather than P limitation.

Understanding the stoichiometric effect of nutrient inputs on a recipient ecosystem must include consideration of spatial scale. At a watershed scale, nutrient inputs can bring about a different biotic community with a higher nutrient content, as opposed to the effect on the nutrient content of a particular individual taxon. At a spatial scale of the 0.1–10 ha typically occupied by individual seabird colonies, terrestrial plant foliage is typically more N-rich than non-seabird site plants and often reflects an altered plant community composition (Mulder *et al.* 2011). Similarly, anthropogenic enrichment of stream systems with nutrients typically decreases biota C : N (Sistla *et al.* 2015).

At a taxon level, the extent to which nutrient inputs affect stoichiometry depends on the taxon's physiology (Frost *et al.* 2005; Kendrick and Benstead 2013), with the capacity of organisms to tolerate (or respond to) stoichiometric imbalances varying widely. Nevertheless, an individual animal typically moderates changes in dietary stoichiometry by either excretion (Zhang *et al.* 2014; Moody *et al.* 2015) or diet choice (Simpson and Raubenheimer 1993; Sperfeld *et al.* 2017). In forest soil invertebrates at a Westland petrel (*P. westlandica*) colony, a 15% enhancement of terrestrial amphipod (Talitridae) C : N accompanied a 2.5-fold enrichment in leaf C : N (Hawke and Holdaway 2005; Hawke *et al.* 2017). Small and Pringle (2010) reported that a 50-fold geologically derived enhancement in dissolved reactive P was accompanied by a doubling in consumer C : P ratios in taxonomically similar insect assemblages from a Costa Rican stream system.

C and N incorporation from marine sources into terrestrial and stream systems are readily identified using the stable isotope ratios $^{13}C:^{12}C$ (reported as $\delta^{13}C$) and $^{15}N:^{14}N$ ($\delta^{15}N$), because $\delta^{13}C$ and $\delta^{15}N$ values are comparatively high in marine systems. However, investigations of seabird input effects on nutrient content and stoichiometry have mostly focused on terrestrial plant foliage. In the present study we tested the hypothesis that breeding Westland petrels will decrease C : N ratios in stream biota assemblages where substantial marine N input had previously been inferred from $\delta^{15}N$ measurements (Harding *et al.* 2004).

Materials and methods

This research was conducted with permission from the New Zealand Department of Conservation (Permit number WC-26300-FAU) and the University of Canterbury Animal Ethics Committee (Permit 2014/09R).

Study area

The Westland petrel is a medium to large burrowing seabird endemic to New Zealand, with a small but stable population of 3000–5000 breeding pairs (Waugh *et al.* 2006; Wood and Otley 2013). Westland petrels breed in the austral winter and are the only substantive remnant of formerly extensive forest seabird breeding throughout mainland New Zealand (Worthy and Holdaway 2002). Colonies are dispersed unevenly across 700 ha close to ridgelines within four deeply dissected forested catchments in the foothills of the Paparoa Range on the West Coast (South Island), the colonies themselves occupying 10% of this area (Wood and Otley 2013; Fig. 1; Table 1). The colonies are not static, and individual colonies move around the landscape on a time-scale of centuries following earthquakes and major storms (Hawke 2004; Waugh *et al.* 2015). The soil in these colonies is highly P rich, the N : P mass ratio in the soil to 60-cm depth at a Westland petrel colony in the Scotchman Creek catchment ranging from 4.3 : 1 (based on total soil P) to 5.1 : 1 (based on soil P in excess of parent material contributions; Hawke 2005).

Harding *et al.* (2004) investigated two streams with Westland petrels (Scotchman and Liddys creeks) and three reference (non-seabird colony) streams (Canoe, Deverys and Maher creeks). Scotchman and Liddys creek catchments each have an order of magnitude more burrows than the other two catchments with Westland petrels (Table 1). Harding *et al.* (2004) reported 28–38% marine N in four components of seabird stream biota (leaves, moss, mayflies as representative primary consumers, and predatory insects), but noted some ambiguity about $\delta^{15}N$ values from the reference streams because of potential legacy effects from former seabird sites or possibly to wandering agricultural animals. Rather than simply calculating C : N ratios from this dataset, we included all known streams with Westland petrels and all common elements of stream biota. In addition to the only four streams with seabirds (Scotchman, Liddys, Waiwhero and Hibernia creeks), we chose four nearby non-seabird colony streams (Canoe, Deverys, Maher and Gaza creeks; Wood and Otley 2013). The eight streams differed to some extent in mean annual flow (Table 1), although all had similar stream communities and history, geology (typically sandstones), climatic conditions (annual rainfall ~2500 mm; mean annual temperature ~11°C) and riparian plant communities (regenerating temperate mixed podocarp rainforest; Don 1986).

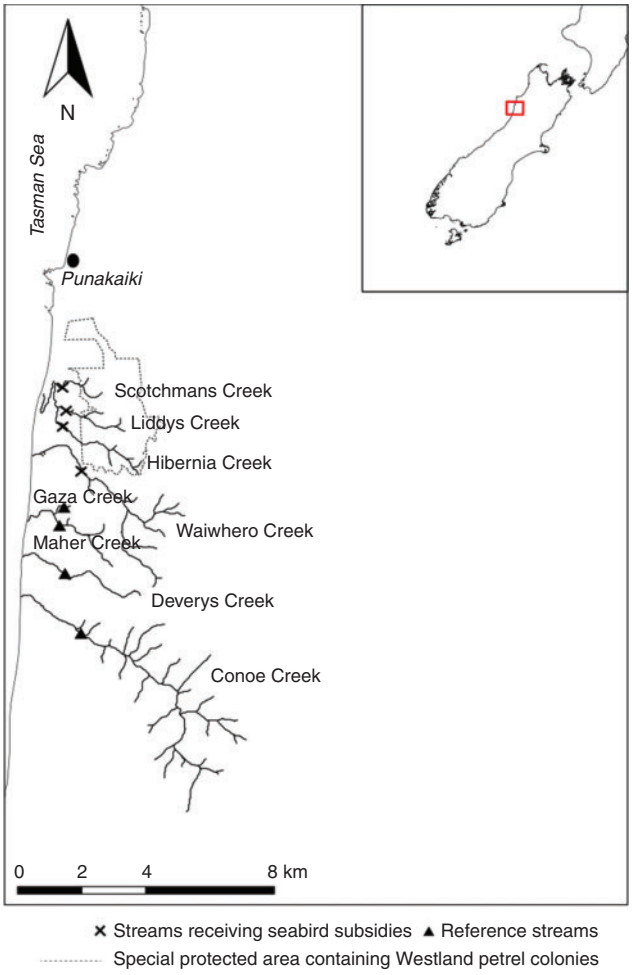

Fig. 1. Study area, showing the four stream catchments hosting Westland petrels and the four reference streams.

Table 1. Mean annual flow, number of seabird burrows and distance from the sampling site to the colony for streams receiving seabird subsidies and reference streams (no seabird subsidies)

Mean annual flow was estimated according to Leathwick *et al.* (2010). Seabird burrows and distance from the sampling site to the colony were taken from Wood and Otley (2013). NA, not applicable

Creek	Mean annual flow (m^3 s^{-1})	Number of seabird burrows	Distance from sampling site to colony (m)
Canoe	0.70	0	NA
Deverys	0.075	0	NA
Gaza	0.0088	0	NA
Maher	0.084	0	NA
Hibernia	0.10	86	2200
Liddys	0.073	2113	1600
Scotchman	0.045	5520	800
Waiwhero	0.30	241	900

There was no agricultural activity in any of the stream catchments upstream of our sampling sites, and only a few isolated dwellings. Stream water chemistry in the study area was dominated by sea salt inputs, except for Ca (D. J. Hawke, unpubl. data). Ca concentrations in seabird streams are higher than non-seabird streams, potentially coleached with nitrate (Likens *et al.* 1998) or directly from seabird guano.

Field methods

We sampled a single reach on each stream, based on results showing negligible differences in macroinvertebrate $\delta^{15}N$ along the reach of Scotchman Creek (Harding *et al.* 2004). At each stream, benthic invertebrates from collector–browser, filter feeder and predator functional feeding groups (Cummins and Klug 1979) were collected using a kick net (mesh 500 µm). Fish (the top predators in these systems) were trapped and riparian spiders (two to four at each site) were hand collected. Freshwater reptiles, amphibians and freshwater mammals are all absent from these systems. Coarse particulate organic matter (>0.5 mm) was collected from streambed debris. Algal mass was scraped from five randomly selected inorganic streambed substrates (e.g. boulders and cobbles). A single composite soil sample was collected comprising three randomly selected cores (15 × 4 cm) taken 5 m apart either side of each stream, and ~1 m from the stream wetted edge. Approximately 50 g (dry weight) of riparian vegetation (live leaves and twigs) representing a selection of plant species was collected within 1 m of the wetted edge. All sampling took place between December 2013 and April 2014.

Water sampling for nitrate-N, dissolved reactive P and pH took place as part of a separate water quality project (D. J. Hawke, unpub. data). We chose a subset of two seabird streams (Scotchman and Hibernia creeks) and two non-seabird streams (Deverys and Maher creeks), the smaller number of streams being chosen to expedite analysis within our field time constraints. Samples (500 mL) were filtered (ashed Whatman GF/F, GE Healthcare, Little Chalfont, Buckinghamshire, UK) and pH measured immediately on site, and chemical analysis (by spectrophotometry) completed at the field base within 2–3 h of sample collection to minimise storage artefacts. The pH measurements used a combination electrode, with a prior two-point calibration offsite followed by a one-point calibration onsite.

Laboratory analysis

Invertebrates were identified to the lowest identifiable taxonomic level within functional feeding groups, and their guts removed to prevent contamination by ingested food materials. Abdominal muscle was dissected from individual fish that had been identified to the species level. Samples were dried at 60°C within a few days of collection, then ground to a fine powder using a ball mill or agate mortar and pestle. Smaller stream invertebrates were combined as required to provide sufficient material for analysis (1 mg); no attempt was made to distinguish between instars, given their similarity.

Total C, total N and the N stable isotope number ratios ($^{15}N:^{14}N$; expressed as $\delta^{15}N$ according to Coplen 2011) were measured from duplicates of finely ground samples using a ThermoFinnigan DeltaPlus continuous flow isotope ratio mass spectrometer (Bremen, Germany) at the University of Canterbury using a two-point normalisation relative to certified reference materials Values for $^{13}C:^{12}C$ (expressed as $\delta^{13}C$) were

also determined, but seabird and reference stream data overlapped. The standard deviations of repeated analyses of an internally calibrated acetanilide control standard were 0.3‰ (δ^{15}N; mean value −0.93‰), 3.7% C (mean value 71.5%) and 0.7% N (mean value 10.1%). C : N ratios (mass : mass) were generated as part of the isotopic analysis.

Stream water nitrate concentrations were determined using the ultraviolet (UV) absorbance method of Armstrong (1963), although the 'compositional effects' referred to by Moorcroft *et al.* (2001) mean that the results can be regarded as indicative only. Dissolved reactive P concentrations were determined using the manual colorimetric method of Murphy and Riley (1962). Both analyses used a Shimadzu UV mini-1240 spectrophotometer (Kyoto, Japan).

Data analysis and display

Nitrogen isotope ratios typically respond to both trophic level and N source, whereas the C : N ratio expresses an organism's nutritional state. To display δ^{15}N values and C : N ratios of the numerous taxa and stream elements in an easy-to-understand way and to visualise the niche spaces occupied in our study system, we plotted δ^{15}N–C : N convex hulls for all taxa from the seabird and non-seabird reference streams. Two-dimensional biplot geometries provide useful numerical representations of ecosystems and their ecological niches, and the convex hull is the simplest geometric form that encloses the data for all the components of a particular ecosystem (Layman *et al.* 2007; Newsome *et al.* 2007). The values for the vertices of the convex hulls came from the means of each taxon or stream element from seabird and reference streams.

Data analysis involved: (1) confirmation of the seabird effect on stream biota δ^{15}N reported by Harding *et al.* (2004); (2) evaluation of the effect of seabirds on C : N ratio; and (3) evaluation of the relationship between stream biota δ^{15}N and their C : N ratios. To confirm the seabird effect on δ^{15}N, we used a paired *t*-tests to compare mean δ^{15}N values from the three stream invertebrate taxa (*Deleatidium* spp., *Coloburiscus* spp., *Archichauliodes diversus*) and a fish (*Gobiomorphus huttoni*) collected from at least two seabird and two reference streams, plus benthic algae, coarse particulate organic matter (CPOM) and riparian plants and soil.

Having confirmed an effect of seabirds on δ^{15}N, we ran *t*-tests separately for individual ecosystem components for C : N *v.* treatment (seabirds *v.* no seabirds) at a family error rate of $\alpha = 0.05$. These tests were controlled for multiplicity by correcting the critical *P*-value using the Bonferroni correction to make the post-correction critical P value $= 0.00625$ (i.e. $\alpha = 0.05 \div 8$ *t*-tests). We did not have enough explanatory power to control for other variables in the *t*-tests.

Finding no stoichiometric response to Westland petrels at an individual ecosystem component level, we analysed C : N against δ^{15}N in a linear mixed effects model (R ver. 3.1.1; R Foundation for Statistical Computing, Vienna, Austria) comparing seabird streams with reference streams. The model (R package lme4, ver. 1.1–7, Bates *et al.* 2015) assumed: (1) random ecosystem component intercepts to account for interspecific differences, noting that taxon size independently predicts neither chitin content (Lease and Wolf 2010) nor trophic niche (Schneider *et al.* 2004); and (2) site intercepts to account for background variability in sites (Bates *et al.* 2015). Subsidy intensity was accounted for using three factors: current burrow numbers (Wood and Otley 2013), a dilution factor (mean annual flow) and distance from the current colony to sampling site. Distance from colony to sampling site was included because of potential changes to stoichiometry occurring through additional processing (Levi *et al.* 2013; Sitters *et al.* 2015). The data for the resulting ecosystem model were analysed at a family error rate of $\alpha = 0.05$ and C : N ratios were log transformed to fit the assumptions of a linear model. For each ecosystem component, increasing δ^{15}N values were assumed to indicate a greater incorporation of seabird N.

Results

Stream water nitrate-N concentrations were higher in the two seabird streams (Scotchman and Hibernia creeks, 86 and 67 μM respectively) than in the two non-seabird streams sampled (Maher and Deverys creeks, 45 and 14 μM respectively). Dissolved reactive P concentrations overlapped (Scotchman and Hibernia creeks, 0.36 and 0.76 μM respectively; Maher and Deverys creeks, 0.41 and 0.15 μM respectively). The pH values were slightly more acidic in the seabird than non-seabird streams (6.42, 6.52, 6.69 and 6.70 in Scotchman, Hibernia, Maher and Deverys creeks respectively).

Seabird stream biota δ^{15}N values were significantly higher than those for reference streams (paired $t = 9.047$, $n = 8$, $P = 0.008$; Fig. 2), consistent with the results reported by Harding *et al.* (2004). The 95% confidence interval of the difference was 1.9–3.2‰. The δ^{15}N–C : N convex hulls from seabird and reference streams were both trapezoid in shape; the seabird stream convex hull centroid (δ^{15}N, 4.8‰) lay 2.9–7.8‰ lower than Westland petrel guano and colony soil and foliage δ^{15}N values (Fig. 2). Unlike the δ^{15}N–C : N convex hulls, the δ^{13}C–C : N data from seabird and reference streams showed no marine isotope effect, largely overlying each other in both individual taxa and in the convex hulls (Fig. S1 in the Supplementary material). Further analysis of the carbon isotope data was therefore not pursued.

Although mean δ^{15}N values from the 11 taxa (plus CPOM and riparian plant foliage and soil) were consistently higher in seabird streams, overall C : N ratios overlapped (Fig. 2, S2–S3 in the Supplementary material). Similarly, the general linear model that allowed for burrow numbers and other catchment variables showed no significant effect of the presence of seabirds on mean C : N ratios (Fig. 3). However, none of the effects were statistically significant. Conversely, when the response of the C : N ratio to δ^{15}N was tested on an ecosystem scale (i.e. when the individual ecosystem components were considered random effects in the analysis), C : N ratios decreased significantly as δ^{15}N increased ($F = 6.7260$, $P = 0.021$; Fig. S4 in the Supplementary material).

Discussion

Our study showed no effect of seabird subsidies on stream biota C : N ratios even though seabirds provide 28–38% of the N in stream biota in Scotchman and Liddys creeks (Harding *et al.* 2004). The absence of a seabird effect on C : N ratios suggests that the taxa in our system buffered the allochthonous N from

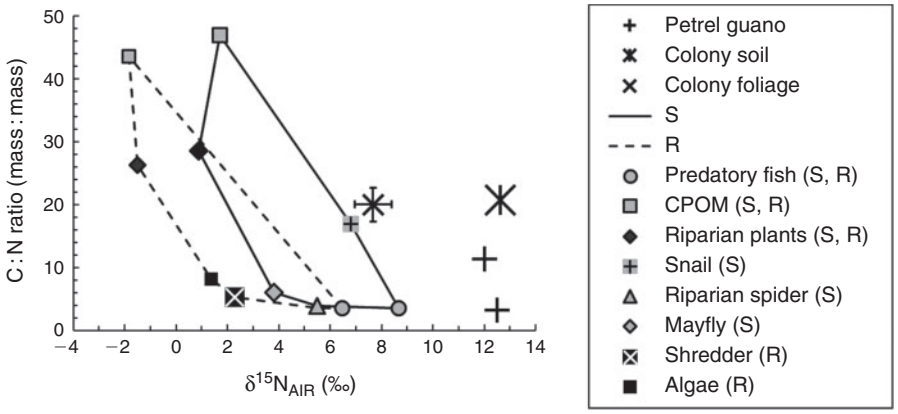

Fig. 2. $\delta^{15}N$ and C : N convex hulls for seabird (S) and reference (R) streams, based on mean values for all taxa collected. Contextual data sources are Hawke (2005), Hawke and Vallance (2015) and Hawke *et al.* (2017). Convex hull vertex abbreviations and ecosystem elements as follows: CPOM, in-stream coarse particulate organic matter; predatory fish, *Gobiomorphus huttoni*; snail, *Potamopyrgus*; riparian spider, *Dolomedes* spp.; shredder, *Coloburiscus* sp.; mayfly, *Deleatidium* spp. The ecosystem elements enclosed within the convex hulls are shown in Fig. S2.

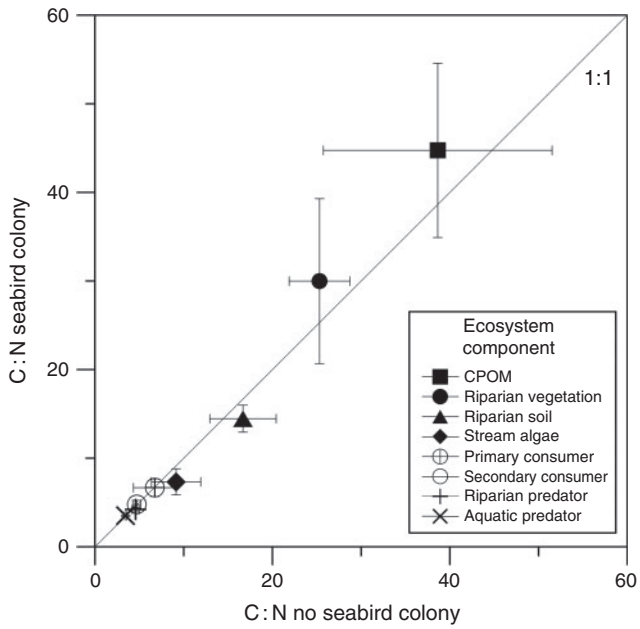

Fig. 3. Mean (±s.d.) C : N ratios for streams with and without seabird colonies for ecosystem components in west coast streams. Anything above or below the 1 : 1 line indicates a difference in the C : N ratio between the two treatments (i.e. streams with *v.* without seabird colonies). Riparian soil, riparian top soil; CPOM, in-stream coarse particulate organic matter; stream algae, biofilm; primary consumer, mayfly *Deleatidium* spp.; secondary consumer, Megaloptera *Archichauliodes diversus*; aquatic predator, redfin bully *Gobiomorphus huttoni*; riparian predator, spider *Dolomedes* spp.

seabirds. Consequently, the calculated seabird-derived N simply replaced non-marine N in stream biota affected by seabirds.

An alternative explanation to physiological buffering of the extra nutrients is that our study streams are light limited rather than nutrient limited. In this scenario, the dense forest cover that dominates the mostly low-order streams sampled in our study overwhelms any nutrient effect on primary production. This possibility is also consistent with the similarity we found in a single sampling of dissolved nutrients. However, the streams in our study had abundant CPOM as an alternative C source for stream animals, a consequence of the same dense forest cover that putatively generated light limitation. Furthermore, colimitation predominates in most systems (Sperfeld *et al.* 2012), of which light is one important factor. Harpole *et al.* (2011) proposed three generalised pathways for colimitation, two being especially relevant here: (1) 'independent colimitation', where small-scale limitation by different single factors appears as colimitation at a larger scale; and (2) 'serial colimitation', where different single factors limit through a time sequence. These types of colimitation affecting benthic algae were recently demonstrated for light and nutrients in forested streams broadly similar to those in the present study, at a reach spatial scale and a day–night temporal scale (Warren *et al.* 2017). In the present study, the consumers varied greatly in size, mobility and life-span from 5-mm *Deleatidium* mayfly larvae to 100-mm, long-lived and wide-ranging fish. Although a role for light limitation at some scale as classified above is highly likely, nutrients may also play a part in stream productivity, along with potential physiological effects.

The seabird and reference streams in this study each encompassed $\delta^{15}N$ of 8–9‰ or ~2.5–3 trophic levels, from carbon-rich CPOM to protein-rich predatory fish. The strong inverse relationship between C : N ratios and $\delta^{15}N$ that we found in our combined seabird and reference stream data matches decreases in C : N ratios with increasing trophic level found in streams elsewhere (Cross *et al.* 2003; Evans-White *et al.* 2005). The decrease in C : N ratios with increasing trophic level demonstrates an ecosystem-scale loss of C relative to N, a process well known in soils as leaf litter decomposes (Berg and Laskowski 2006). The great surprise of the present study is that seabird nutrients did not appear to disturb this process in streams, even though the level of marine N incorporation in stream biota is large (Harding *et al.* 2004) and Westland petrel colony soil

microbes are carbon limited due to nutrient saturation (Hawke and Vallance 2015). Similarly, seabird nutrients did not disturb the δ^{15}N–C : N convex hulls, apart from the seabird-driven shift in δ^{15}N. This is despite the sensitivity of convex hulls to changes over different time-scales in the isotopic signatures of their basal resources, such as ammonia and nitrate (Layman *et al.* 2012).

Our analysis disregarded the potential effect of fluctuations in petrel populations over a range of time-scales, and associated legacy effects on nutrient fluxes to streams. Sections of colonies are lost in response to storms of varying intensities and to earthquakes, re-establishing later when soil depth allows burrowing (Hawke and Powell 1995; Hawke 2004; Waugh *et al.* 2015). Superimposed on these entirely natural events is the extirpation of most of the seabirds that occupied our general study area following human settlement sometime after 1320 CE (Worthy and Holdaway 1993; Holdaway *et al.* 2014).

Given that nutrients remain in the soil after the seabirds have gone (Mizutani *et al.* 1991; Hawke and Powell 1995), legacy effects on fluxes to streams are a very real possibility. However, the readily detectable legacy elements in soil do not necessarily imply corresponding legacy fluxes to streams. As noted elsewhere (Hawke and Newman 2004), allochthonous nutrients can only accumulate in soil to the extent that soil binding capacity allows. Because of the high level of seabird nutrient input and finite binding capacity in soil, nutrients turn over quickly, a decadal residence time being calculated for the Scotchman Creek system (Hawke 2005). However, once seabird inputs cease at a particular site, losses will diminish quickly as nutrient concentrations decrease to match the availability of soil binding sites. Therefore, these legacy fluxes to streams will become increasingly difficult to identify.

Because the catchments in the general study region were substantially affected by seabirds (Worthy and Holdaway 1993), testing potential legacy effects by studies outside the area presently occupied by Westland petrels would be of questionable validity. Another way of resolving the potential legacy effect would be an *in situ* nutrient limitation experiment. Unfortunately, we have already unsuccessfully attempted these experiments, which are problematic in our study area given intense rain storms typically occurring numerous times a year.

A challenge faced by field-based surveys such as ours is that stoichiometric effects can be masked by variability in size distributions and invertebrate ontogeny across multiple taxa (Halvorson and Small 2016). Halvorson and Small (2016) argued that experimental manipulations on single taxa comprise the only valid approach for assessing nutrient addition effects on stoichiometric ratios. Although laboratory experiments provide an excellent way of assessing the stoichiometric flexibility of individual taxa, there is also a need to know what happens when these taxa are assembled within ecosystems. However, the stoichiometric approach to the assessment of ecological limitation is itself coming under pressure as the complexity of molecular responses to limitation or enrichment become better known (Wilder and Jeyasingh 2016). Unfortunately, measurement of physiologically relevant biomolecules remains time consuming and relatively complex, whereas C and N data are included with stable isotope results generated by high-throughput automated instruments. It is not practical to analyse all samples for all conceivable analytes. Rather, we suggest that observational field studies such as ours serve as a basis for more directed chemical and ecological analysis, including in vitro experiments.

Our study addressed the physiological question of whether nutrient inputs from Westland petrels could affect the stoichiometry of stream organisms, similar to that observed in plant foliage and soil invertebrates within the Westland petrel breeding colonies alongside increases in δ^{15}N values (Hawke and Holdaway 2005). Although disruptions to stoichiometry appear confined to the terrestrial environment of the petrel colony, our study leaves open the question of Westland petrel effects on ecological measures such as in-stream productivity and biomass. Studies on salmonid spawning habitats have demonstrated complex interactions between the amount of marine nutrient loading and stream productivity and biomass (Wipfli *et al.* 1999; Collins *et al.* 2015; Samways and Cunjak 2015). Although mass balance calculations (Hawke 2005) demonstrate that Westland petrel-derived nutrient fluxes to streams will be substantial, the likely moderation of these inputs by a combination of soil drainage and overland flow suggests that interactions in Westland petrel streams will be just as complex as those occurring in the salmon streams of the Pacific North-west and the North-east Atlantic.

Conclusions

Ecological stoichiometry is a useful theoretical framework linked to ecological processes such as reproduction and nutrient cycling. Although nutrient enrichment effects on C : N and C : P ratios have been reported in a wide range of terrestrial and stream invertebrates, we found that the presence of Westland petrels did not measurably affect the C : N ratios of stream biota. Consequently, our results demonstrate that elevated ecosystem δ^{15}N values from marine-derived N do not necessarily lead to direct changes in stoichiometry. Although stable isotope measurements provide a useful indication of marine N movement into a recipient ecosystem, a comprehensive assessment of the effect of seabirds on stream ecosystems should include measurements of ecologically significant quantities, such as biomass (and associated mass balance measurements) and productivity. Given that stoichiometry is a convenient proxy for more complex molecular-scale effects, these field measurements could usefully be complemented by laboratory experiments focusing on essential biomolecules, such as essential fatty acids and essential amino acids.

Conflict of interest

The authors declare that they have no conflicts of interest.

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References

Armstrong, F. A. J. (1963). Determination of nitrate in water by ultraviolet spectrophotometry. *Analytical Chemistry* **35**, 1292–1294. doi:10.1021/AC60202A036

Balaine, N., Clough, T. J., Beare, M. H., Thomas, S. M., and Meenken, E. D. (2016). Soil gas diffusivity controls N_2O and N_2 emissions and their ratio. *Soil Science Society of America Journal* **80**, 529–540. doi:10.2136/SSSAJ2015.09.0350

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**(1), 1–48. doi:10.18637/JSS.V067.I01

Berg, B., and Laskowski, R. (2006). 'Litter Decomposition: a Guide to Carbon and Nutrient Turnover.' (Academic Press: Burlington, VT, USA.)

Burger, A. E., Lindeboom, H. J., and Williams, A. J. (1978). The mineral and energy contributions of guano of selected species of birds to the Marion Island terrestrial ecosystem. *South African Journal of Antarctic Research* **8**, 59–70.

Collins, S. F., Marcarelli, A. M., Baxter, C. V., and Wipfli, M. S. (2015). A critical assessment of the ecological assumptions underpinning compensatory mitigation of salmon-derived nutrients. *Ecological Management* **56**, 571–586.

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry* **25**, 2538–2560. doi:10.1002/RCM.5129

Cross, W. F., Benstead, J. P., Rosemond, A. D., and Wallace, J. B. (2003). Consumer–resource stoichiometry in detritus-based streams. *Ecology Letters* **6**, 721–732. doi:10.1046/J.1461-0248.2003.00481.X

Cummins, K. W., and Klug, M. J. (1979). Feeding ecology of stream invertebrates. *Annual Review of Ecology and Systematics* **10**, 147–172. doi:10.1146/ANNUREV.ES.10.110179.001051

Don, G. L. (1986). A biological survey of Barrytown Flat. Environmental impact assessment report. Bioresearches Ltd, Christchurch, New Zealand.

Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**, 1135–1142. doi:10.1111/J.1461-0248.2007.01113.X

Evans-White, M. A., Stelzer, R. S., and Lamberti, G. A. (2005). Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. *Freshwater Biology* **50**, 1786–1799. doi:10.1111/J.1365-2427.2005.01455.X

Fairhurst, G. D., Bond, A. L., Hobson, K. A., and Ronconi, R. A. (2015). Feather-based measures of stable isotopes and corticosterone reveal a relationship between trophic position and physiology in a pelagic seabird over a 153-year period. *The Ibis* **157**, 273–283. doi:10.1111/IBI.12232

Frainer, A., Jabiol, J., Gessner, M. O., Bruder, A., Chauvet, E., and McKie, B. G. (2016). Stoichiometric imbalances between detritus and detritivores are related to shifts in ecosystem functioning. *Oikos* **125**, 861–871. doi:10.1111/OIK.02687

Frost, P. C., Evans-White, M. A., Finkel, Z. V., Jensen, T. C., and Matzek, V. (2005). Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos* **109**, 18–28. doi:10.1111/J.0030-1299.2005.14049.X

Furness, R. M. (1991). The occurrence of burrow nesting among birds and its influence on soil stability. Symposium of the Zoological Society of London 63, 53–67.

Halvorson, H. M., and Small, G. E. (2016). Observational field studies are not appropriate tests of consumer stoichiometric homeostasis. *Freshwater Science* **35**, 1103–1116. doi:10.1086/689212

Harding, J. S., Hawke, D. J., Holdaway, R. N., and Winterbourn, M. J. (2004). Incorporation of marine-derived nutrients from petrel breeding colonies into stream food webs. *Freshwater Biology* **49**, 576–586. doi:10.1111/J.1365-2427.2004.01210.X

Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E. S., Elser, J. J., Gruner, D. S., Hillebrand, H., Shurin, J. B., and Smith, J. E. (2011). Nutrient co-limitation of primary producer communities. *Ecology Letters* **14**, 852–862. doi:10.1111/J.1461-0248.2011.01651.X

Hawke, D. J. (2004). Maximum possible age of a petrel breeding colony near Punakaiki (south Island, New Zealand) from radiocarbon and stable isotope analysis of soil. *Journal of the Royal society of New Zealand* **34**, 1–7.

Hawke, D. J. (2005). Soil P in a forested seabird colony: inventories, parent material contributions, and N : P stoichiometry. *Australian Journal of Soil Research* **43**, 957–962. doi:10.1071/SR05075

Hawke, D. J., and Condron, L. M. (2014). Mobilisation of recalcitrant soil nutrient fractions supports foliar nitrogen to phosphorus homeostasis in a seabird soil. *Plant and Soil* **385**, 77–86. doi:10.1007/S11104-014-2228-0

Hawke, D. J., and Holdaway, R. N. (2005). Avian assimilation and dispersal of carbon and nitrogen brought ashore by breeding Westland petrels (*Procellaria westlandica*): a stable isotope study. *Journal of Zoology* **266**, 419–426. doi:10.1017/S0952836905007065

Hawke, D. J., and Newman, J. (2004). Inventories and elemental accumulation in peat soils of forested seabird breeding islands, southern New Zealand. *Australian Journal of Soil Research* **42**, 45–48. doi:10.1071/SR03107

Hawke, D. J., and Powell, H. K. J. (1995). Soil solution chemistry at a Westland petrel breeding colony, New Zealand: palaeoecological implications. *Australian Journal of Soil Research* **33**, 915–924. doi:10.1071/SR9950915

Hawke, D. J., and Vallance, J. R. (2015). Microbial carbon distribution in samples of seabird forest soil: implications for leaf litter cycling. *Pedobiologia* **58**, 33–39. doi:10.1016/J.PEDOBI.2015.01.002

Hawke, D. J., Cranney, O. R., Horton, T. W., Bury, S. J., Brown, J. C. S., and Holdaway, R. N. (2017). Foliar and soil N and $\delta^{15}N$ as restoration metrics at Pūtaringamotu Riccarton Bush, Christchurch city. *Journal of the Royal Society of New Zealand* **47**, 319–335. doi:10.1080/03036758.2017.1281319

Hessen, D. O., Elser, J. J., Sterner, R. W., and Urabe, J. (2013). Ecological stoichiometry: an elementary approach using basic principles. *Limnology and Oceanography* **58**, 2219–2236. doi:10.4319/LO.2013.58.6.2219

Hicks, B. J., Wipfli, M. S., Lang, D. W., and Lang, M. E. (2005). Marine-derived nitrogen and carbon in freshwater-riparian food webs of the Copper River Delta, southcentral Alaska. *Oecologia* **144**, 558–569. doi:10.1007/S00442-005-0035-2

Holdaway, R. N., Allentoft, M. E., Jacomb, C., Oskam, C. L., Beavan, N. R., and Bunce, M. (2014). An extremely low-density human population exterminated New Zealand moa. *Nature Communications* **5**, 5436. doi:10.1038/NCOMMS6436

Janetski, D. J., Chaloner, D. T., Tiegs, S. D., and Lamberti, G. A. (2009). Pacific salmon effects on stream ecosystems: a quantitative synthesis. *Oecologia* **159**, 583–595. doi:10.1007/S00442-008-1249-X

Kendrick, M. R., and Benstead, J. P. (2013). Temperature and nutrient availability interact to mediate growth and body stoichiometry in a detritivorous stream insect. *Freshwater Biology* **58**, 1820–1830. doi:10.1111/FWB.12170

Kerpel, S. M., Soprano, E., and Moreira, G. R. P. (2006). Effect of nitrogen on *Passiflora suberosa* L. (Passifloraceae) and consequences for larval performance an oviposition in *Heliconius erato phyllis* (Fabricius) (Lepidoptera: Nymphalidae). *Neotropical Entomology* **35**, 192–200. doi:10.1590/S1519-566X2006000200006

Layman, C. A., Arrington, D. A., Montaña, C. G., and Post, D. M. (2007). Can stable isotope ratios provide quantitative measures of trophic diversity within food webs? *Ecology* **88**, 42–48. doi:10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J.,

Yeager, L. A., Post, D. M., and Bearhop, S. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews of the Cambridge Philosophical Society* **87**, 545–562. doi:10.1111/J.1469-185X.2011.00208.X

Lease, H. M., and Wolf, B. O. (2010). Exoskeletal chitin scales isometrically with body size in terrestrial insects. *Journal of Morphology* **271**, 759–768.

Leathwick, J. R., West, D., Gerbeaux, P., Kelly, D., Robertson, H., Brown, D., Chadderton, W. L., and Ausseil, A. G. (2010). 'Freshwater Ecosystems of New Zealand (FENZ) Geodatabase. Version 1: User Guide.' (Department of Conservation: Wellington, New Zealand.)

Levi, P. S., Tank, J. L., Tiegs, S. D., Chaloner, D. T., and Lamberti, G. A. (2013). Biogeochemical transformation of a nutrient subsidy: salmon, streams, and nitrification. *Biogeochemistry* **113**(1-3), 643–655. doi:10.1007/S10533-012-9794-0

Likens, G. E., Driscoll, C. T., Buso, D. C., Siccama, T. G., Johnson, C. E., Lovett, G. M., Fahey, T. J., Reiners, W. A., Ryan, D. F., Martin, C. W., and Bailey, S. W. (1998). The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry* **41**, 89–173. doi:10.1023/A:1005984620681

Mizutani, H., Kabaya, Y., Moors, P. J., Spier, T. W., and Lyon, G. L. (1991). Nitrogen isotope ratios identify deserted seabird rookeries. *The Auk* **108**, 960–964.

Moody, E. K., Corman, J. R., Elser, J. J., and Sabo, J. L. (2015). Diet composition affects the rate and N : P ratio of fish excretion. *Freshwater Biology* **60**, 456–465. doi:10.1111/FWB.12500

Moorcroft, M. J., Davis, J., and Compton, R. G. (2001). Detection and determination of nitrate and nitrite: a review. *Talanta* **54**, 785–803. doi:10.1016/S0039-9140(01)00323-X

Mulder, C. P. H., Jones, H. P., Kameda, K., Palmborg, C., Schmidt, S., Ellis, J. C., Orrock, J. L., Wait, D. A., Wardle, D. A., Yang, L., Young, H., Croll, D. A., and Vidal, E. (2011). Impacts of seabirds on plant and soil properties. In 'Seabird Islands: Ecology, Invasion, and Restoration'. (Eds C. P. H. Mulder, W. B. Anderson, D. R. Towns, and P. J. Bellingham.) pp. 135–176. (Oxford University Press: New York, NY, USA.)

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**, 31–36. doi:10.1016/S0003-2670(00)88444-5

Naiman, R. J., Bilby, R. E., Schindler, D. E., and Helfield, J. M. (2002). Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. *Ecosystems* **5**, 399–417. doi:10.1007/S10021-001-0083-3

Newsome, S. D., Martinez del Rio, C., Bearhop, S., and Phillips, D. L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and Evolution* **5**, 429–436. doi:10.1890/060150.1

Polis, G. A., Power, M. E., and Huxel, G. R. (Eds) (2004). 'Food Webs at the Landscape Level.' (Chicago University Press: Chicago, IL, USA.)

Richardson, S., Peltzer, D. A., McGlone, M. S., and Parfitt, R. L. (2004). Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia* **139**, 267–276. doi:10.1007/S00442-004-1501-Y

Samways, K. M., and Cunjak, R. A. (2015). Increases in benthic community production and metabolism in response to marine-derived nutrients from spawning Atlantic salmon (*Salmo salar*). *Freshwater Biology* **60**, 1647–1658. doi:10.1111/FWB.12597

Sardans, J., Rivas-Ubach, A., and Peñuelas, J. (2012). The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationship with organismic lifestyle and ecosystem structure and function: a review and perspectives. *Biogeochemistry* **111**, 1–39. doi:10.1007/S10533-011-9640-9

Schneider, K., Migge, S., Norton, R. A., Scheu, S., Langel, R., Reineking, A., and Maraun, M. (2004). Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios ($^{15}N/^{14}N$). *Soil Biology & Biochemistry* **36**, 1769–1774. doi:10.1016/J.SOILBIO.2004.04.033

Simpson, S. J., and Raubenheimer, D. (1993). A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **342**, 381–402. doi:10.1098/RSTB.1993.0166

Sistla, S. A., Appling, A. P., Lewandowska, A. M., Taylor, B. N., and Wolf, A. A. (2015). Stoichiometric flexibility in response to fertilization along gradients of environmental and organismal nutrient richness. *Oikos* **124**, 949–959. doi:10.1111/OIK.02385

Sitters, J., Atkinson, C. L., Guelzow, N., Kelly, P., and Sullivan, L. L. (2015). Spatial stoichiometry: cross-ecosystem material flows and their impact on recipient ecosystems and organisms. *Oikos* **124**, 920–930. doi:10.1111/OIK.02392

Small, G. E., and Pringle, C. M. (2010). Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a neotropical stream. *Oecologia* **162**, 581–590. doi:10.1007/S00442-009-1489-4

Smith, J. L., Mulder, C. P. H., and Ellis, J. C. (2011). Seabirds as ecosystem engineers: nutrient inputs and physical disturbance. In 'Seabird Islands. Ecology, Invasion and Restoration'. (Eds C. P. Mulder, W. B. Anderson, D. R. Towns, and P. J. Bellingham.) pp. 27–55. (Oxford University Press: New York, NY, USA.)

Sperfeld, E., Martin-Creuzberg, D., and Wacker, A. (2012). Multiple resource limitation theory applied to herbivorous consumers: Liebig's minimum rule vs. interactive co-limitation. *Ecology Letters* **15**, 142–150. doi:10.1111/J.1461-0248.2011.01719.X

Sperfeld, E., Wagner, N. D., Halvorson, H. M., Malishev, M., and Raubenheimer, D. (2017). Bridging ecological stoichiometry and nutritional geometry with homeostasis concepts and integrative models of organism nutrition. *Functional Ecology* **31**, 286–296. doi:10.1111/1365-2435.12707

Sudduth, E. B., Perakis, S. S., and Bernhardt, B. S. (2013). Nitrate in watersheds: straight from soils to streams? *Journal of Geophysical Research. Biogeosciences* **118**, 291–302. doi:10.1002/JGRG.20030

Tessier, J. T., and Raynal, D. J. (2003). Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. *Journal of Applied Ecology* **40**, 523–534. doi:10.1046/J.1365-2664.2003.00820.X

Trochine, C., Guerrieri, M., Liboriussen, L., Willems, P., Lauridsen, T. L., Søndergaard, M., and Jeppesen, E. (2017). Factors controlling the stable isotope composition and C : N ratio of seston and periphyton in shallow lake mesocosms with contrasting nutrient loadings and temperatures. *Freshwater Biology* **62**, 1596–1613. doi:10.1111/FWB.12971

Twining, C. W., Brenna, J. T., Hairston, N. G., Jr, and Flecker, A. S. (2016). Highly unsaturated fatty acids in nature: what we know and what we need to learn. *Oikos* **125**, 749–760. doi:10.1111/OIK.02910

Walker, T. W., and Adams, A. F. R. (1958). Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulphur, and organic phosphorus in grassland soils. *Soil Science* **85**, 307–318. doi:10.1097/00010694-195806000-00004

Warren, D. R., Collins, S. M., Purvis, E. M., Kaylor, M. J., and Bechtold, H. A. (2017). Spatial variability in light yields colimitation of primary production by both light and nutrients in a forested stream ecosystem. *Ecosystems* **20**, 198–210. doi:10.1007/S10021-016-0024-9

Waugh, S. M., Doherty, P. F., Freeman, A. N. D., Adam, L., Woods, G. C., Bartle, J. A., and Hedley, G. K. (2006). Demography of Westland petrels (*Procellaria westlandica*), 1995–2003. *Emu* **106**, 219–226. doi:10.1071/MU05065

Waugh, S. M., Poupart, T., and Wilson, K.-J. (2015). Storm damage to Westland petrel colonies in 2014 from Cyclone Ita. *Notornis* **62**, 165–168.

Whitehead, D. C., and Raistrick, N. (1993). The volatilization of ammonia from cattle urine applied to soils as influenced by soil properties. *Plant and Soil* **148**, 43–51. doi:10.1007/BF02185383

Wilder, S. M., and Jeyasingh, P. D. (2016). Merging elemental and macronutrient approaches for a comprehensive study of energy and nutrient flows. *Journal of Animal Ecology* **85**, 1427–1430. doi:10.1111/1365-2656.12573

Wipfli, M. S., Hudson, J., Chaloner, D. T., and Caouette, J. P. (1999). The influence of salmon spawner densities on stream productivity in Southeast Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1600–1611. doi:10.1139/F99-087

Wood, G., and Otley, H. (2013). An assessment of the breeding range, colony sizes and population of the Westland petrel (*Procellaria westlandica*). *New Zealand Journal of Zoology* **40**, 186–195. doi:10.1080/03014223.2012.736394

Worthy, T. H., and Holdaway, R. N. (1993). Quaternary fossil fauna from caves in the Punakaiki area, West Coast, South Island, New Zealand. *Journal of the Royal Society of New Zealand* **23**, 147–254. doi:10.1080/03036758.1993.10721222

Worthy, T. H., and Holdaway, R. N. (2002). 'The Lost World of the Moa: Prehistoric Life of New Zealand.' (Indiana University Press: Bloomington, IN, USA.)

Zhang, Z., Elser, J. J., Cease, A. J., Zhang, X., Yu, Q., Han, X., and Zhang, G. (2014). Grasshoppers regulate N : P stoichiometric homeostasis by changing phosphorus content in their frass. *PLoS One* **9**, e103697. doi:10.1371/JOURNAL.PONE.0103697