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Anthropogenic land-use signals propagate through stream food webs in a California, USA, watershed

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ABSTRACT

Human development of watersheds can change aquatic ecosystems via multiple pathways. For instance, human rural development may add nutrients to ecosystems. We used naturally occurring stable isotopes in stream food webs to investigate how land use affects stream ecosystems across a gradient of land development in the San Lorenzo watershed, California. Road density was used as a proxy for land development. We found that streams in watersheds with higher road densities had elevated concentrations of phosphate and nitrate. Furthermore, algal $\delta^{15}N$ values increased as a function of nitrate concentration, but saturated at approximately 6‰. This saturating pattern was consistent with a two-source mixing model with anthropogenic and watershed sources, fit using Bayesian model fitting. In sites that had >2.6 km roads km^{-2}, anthropogenic sources of N were estimated to represent >90% of the N pool. This anthropogenic N signal was propagated to stream consumers: rainbow trout (*Oncorhynchus mykiss*), signal crayfish (*Pacifasticus leniusculus*), and benthic invertebrate $\delta^{15}N$ were positively correlated with algal $\delta^{15}N$. Even relatively low density rural human land use may have substantial impacts on nutrient cycling of stream ecosystems.

Introduction

Human land-use impacts freshwater ecosystems via multiple pathways, such as through nutrient loading and habitat alteration (Naiman and Turner, 2000). Human activities increase loading of limiting nutrients such as nitrogen (N) and phosphorus (P) (Elser et al., 2007; Holtgrieve et al., 2011), driving cultural eutrophication of aquatic systems (Smith and Schindler, 2009). As hydrologic connectors, streams are recipients of and conduits for these nutrients (Gomi et al., 2002). Thus, streams and their communities can be locally impacted by elevated nutrients but then also propagate nutrients to downstream ecosystems like estuaries and lakes that may be vulnerable to eutrophication (Smith and Schindler, 2009). Consumers in aquatic systems may also be affected by shifting resource bases associated with land-use changes. Although anthropogenic activities can increase nutrient loading to freshwaters via many pathways (Smith and Schindler, 2009), non-point sources of pollution have proven particularly difficult to quantify, monitor, or regulate (Carpenter et al., 1998).

Stable isotopes are increasingly used to investigate how anthropogenic land-use alters aquatic ecosystems. For example, nitrogen stable isotope ratios ($\delta^{15}N$) can identify potential sources of nitrogen as well as inform rates of nutrient transformations (Peterson and Fry, 1987; Robinson, 2001). Nitrogen stable isotopes are commonly used to estimate the mixing of anthropogenic sources of nutrients into food webs and ecosystems. Human sewage is generally enriched relative to other watershed sources. This strong contrast of anthropogenic versus background watershed sources provides the opportunity, for example, to locate non-point sources of sewage pollution (Cabana and Rasmussen, 1996; Steffy and Kilham, 2004; Cole et al., 2006; Kaushal et al., 2006; Leavitt et al., 2006). One potentially insightful approach to examining patterns of stable isotopes is to compare mixing models with different

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potential sources (e.g., Vander Zanden et al., 2005). For example, in a study of streams in an agricultural region, Diebel and Vander Zanden (2009) found that variance in N isotope ratios among stream biota was best explained by inorganic fertilizer application and wetland land cover within the watershed, suggesting the importance of fertilizer-derived nitrate and its removal via denitrification in wetlands. Such approaches may be an important step forward to identify and quantify potential sources of excess nutrients to watersheds (Smith and Schindler, 2009).

The ecological effects of human land use can be illuminated through the study of gradients that span urban to rural developments (McDonnell and Pickett, 1990). In many parts of western North America, people are inhabiting more rural landscapes including the headwaters of watersheds (e.g., Kaushal et al., 2006). Although densities of people may be relatively low in these rural areas, the impacts of such developments on aquatic ecosystems may be large (e.g., Moore et al., 2003; Burtner et al., 2011). Here we used stable isotopes to examine how human land-use alters stream ecosystems in the San Lorenzo watershed, California along a gradient of human land-use intensity. We asked the question: how does human land-use alter patterns of nutrients and isotopes in stream communities?

Materials and methods

Study system

We examined 12 sites within the San Lorenzo River watershed (Santa Cruz County, CA, USA) that spanned a gradient of human land-use intensity. Sites were located on the numerous relatively small (first and second order) streams within the watershed and were part of a larger study (D. B. Herbst, unpublished data). From this larger set of candidate sites, sites were chosen to stratify a gradient in human land-use intensity and minimize differences in gradient and stream size. With two exceptions, sites were located on different streams. Elevations in this coastal watershed range from 979 m to sea level where the San Lorenzo River enters the Monterey Bay. The climate is Mediterranean, with 76–153 cm rain yr^{-1}. Tributaries drain steep soils of weathering granite, schists, marble, and marine deposits consisting of sandstones, shales and mudstones (Herbert, 2009). Most of the watershed is characterized by dense second growth mixed evergreen redwood forest and sparse rural development.

The San Lorenzo watershed has a history of excess anthropogenic nutrient inputs (Ricker et al., 2001). Of particular concern is that excess nutrient pollution has decreased water quality in the downstream lagoon and nearby ocean beaches; high counts of fecal coliform and other indicators of nutrient pollution have led to public warnings that these areas are unsafe for swimming (Ricker et al., 2001). Sources of elevated nitrate levels in San Lorenzo watershed streams include leaking septic and sewer systems, livestock, and urban runoff (Herbert, 2009). Indeed, as of 2000 there were approximately 14,000 septic systems within the 358 km^2 watershed (Herbert, 2009). It is thought that the steep terrain, seasonally high water tables, episodic stream flows, and limited reduction of nitrates through filtration or denitrification in sandy areas, further contribute to elevated nitrate levels in San Lorenzo River watershed streams (Herbert, 2009).

Field study

Each site consisted of a riffle-pool sequence ranging from 40 to 60 m in length. Sites encompassed an anthropogenic gradient of the watershed which ranged from locations with little anthropogenic influence to locations with higher levels of anthropogenic influence such as road crossing and rural development. We used catchment road density (km km^{-2}) as an index of human land-use intensity for each site. At each site, we collected primary producers (periphyton), and consumers (benthic invertebrates; rainbow trout, *Oncorhynchus mykiss*; signal crayfish, *Pacifasticus leniusculus*) for stable isotope analyses and water samples to obtain nutrient concentrations. All sampling was conducted in June 20–26, 2009 at near base stream flow conditions.

Primary producer biomass was characterized by algae (periphyton) scraped from cobbles collected from both a region of slow- and fast-water within the sampling site. Previous work has found that water velocity can influence algal stable isotope signatures (Findlay et al., 1999, 2002). These samples were analyzed for stable isotopes separately. However, our analyses ended up focused on nitrogen isotopes and these were similar (see section "Results"). We thus pooled algal samples for analyses. Algae samples were frozen immediately after collection.

At each site benthic invertebrates were collected by a Surber sample (0.5 mm mesh; sampling to a depth of 10 cm) in both a region of fast and slow stream flow. Samples were preserved in 70% ethanol. We note that preservation in ethanol can slightly alter isotope signatures (shifting $\delta^{13}C$ approximately 1‰ and $\delta^{15}N$ approximately 0.4 ‰; Venturra and Jeppesen, 2009). We did not adjust for this shift because it is likely relatively consistent within invertebrates that have fairly constrained stoichiometry (as opposed to across taxonomic groups with vastly different stoichiometry). Prior to preparation for stable isotope analysis, invertebrates were sorted and identified to family and functional feeding group (filterer, detritivore, herbivore, or predator) according to Merritt et al. (2008). For each functional feeding group present at a site, one or two samples were selected for stable isotope analysis. While samples were run separately, invertebrates from different functional groups were pooled in subsequent analyses because of the lack of replication within functional groups.

Fish and crayfish were collected by three-pass depletion electrofishing. Block nets at the upper and lower extent of each site prevented movement in or out of the site during surveys. Signal crayfish (*Pacifastacus leniusculus*; $n = 35$, approximately three per site) and rainbow trout (*O. mykiss*; $n = 65$, approximately five per site) were selected as focal species as they were the most abundant top aquatic consumers present across the different sampling sites. Orbital carapace length (crayfish) or total fork length (trout) and wet weight (to the nearest 0.1 g) were measured on-site for each sampled organism. Crayfish muscle tissue and rainbow trout caudal fin clips (Hanisch et al., 2010; Heady and Moore, 2013) were collected for stable isotope analysis and immediately frozen.

Algae and benthic invertebrates were oven dried until they reached a constant weight (approximately 48 h at 60 °C), whereas crayfish and trout samples were freeze-dried. To remove ^{13}C-depleted lipids, all consumer samples were flushed with three cycles of petroleum ether at 1200 psi in a Dionex ASE 200 Accelerated Solvent Extraction System. Algae and crayfish tissue samples were ground into a homogenous powder with an agate mortar and pestle. Larger benthic invertebrates were also ground into a fine powder, whereas multiple individuals of the same species were aggregated into one sample for smaller invertebrates. Trout fin clips were left intact. Samples were weighed into 5 mm × 9 mm (algae, mean ± standard deviation = 4800 ± 31 μg) or 3 mm × 5 mm (benthic invertebrates, 589 ± 53 μg; crayfish muscle, 698 ± 30 μg; fish fins, 673 ± 78 μg) tin capsules (Costech Analytical Technologies).

Stable isotope analyses

Sample $\delta^{13}C$ and $\delta^{15}N$ were measured on a Carlo Erba 1108 elemental analyzer coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (University of California, Santa Cruz Stable