**Effects of spatially heterogeneous lakeside development on nearshore biotic communities in a large, deep, oligotrophic lake (Lake Baikal, Siberia)**

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**Keywords**: Lake Baikal, sewage, PPCP, food webs, community ecology, human disturbance

**Abstract (249/250 words)**

Clustered anthropogenic activity along lakeshores can create hot spots of disturbance with ecological consequences. Sewage released from such lakeside development is a type of high impact human disturbance with effects most immediately observed among littoral biota. Lake Baikal, a large, oligotrophic lake, has been experiencing localized sewage pollution from lakeside settlements; increasing filamentous algal abundance has provided evidence that nearshore benthic may be responding to sewage inputs. To test the presence and magnitude of sewage release into Lake Baikal, we surveyed 14 littoral sites along a 40-km transect of the southwestern shore for sewage indicators, including pharmaceuticals and personal care products (PPCPs) and microplastics. To examine benthic community responses, periphyton and macroinvertebrate relative abundance as well as community interactions were assessed at each littoral site. At all sites, PPCPs including caffeine (up to 60 ng/L) and acetaminophen (up to 40 ng/L) were detected in the nearshore water column, and concentrations were related to extent of lakeside development. Benthic invertebrate and periphyton communities also responded to lakeside development. Periphyton and macroinvertebrate stable isotope analysis and essential fatty acid compositions suggested that, despite differences in community composition among sites, the food web structure remained consistent along our study area. Together, our results suggest population hot spots can lead to gradients of human disturbance, thereby creating spatial heterogeneity in nearshore, benthic communities. For Lake Baikal, these results are timely, especially considering growing tourism hot spots (~1.2 million tourists annually) in tandem with developing wastewater management may heighten risk for future environmental consequences.

**Introduction**

The release of treated and untreated wastewater into aquatic ecosystems is a common human disturbance that can introduce pollutants and reshape aquatic ecological communities (Hampton et al. 2011). Nutrients are among the primary pollutants within sewage byproducts (Smith et al. 1999). Although often concentrated within sewage, nutrient inputs can also originate from disparate anthropogenic and environmental sources, thereby obfuscating sewage signals. For example, agricultural runoff (Powers et al. 2016), geological processes - such as melting permafrost (Turetsky et al. 2000), and changes in terrestrial plant communities (Moran et al. 2012) can all increase allochthonous nutrient inputs similar to sewage. Regardless of the nutrients’ source, biological responses to even slight nutrient increases can further confound sewage signals. Benthic primary producers in nearshore water, especially those in oligotrophic lakes, can assimilate nutrients quickly from the water column (e.g., hours), such that deviation in typical water concentrations is not observed (Hadwen and Bunn 2005).

Because nutrients come from numerous non-sewage sources, pollutants consistently associated with human activity, such as pharmaceuticals and personal care products (PPCPs) (Rosi-Marshall and Royer 2012) and microplastics (Barnes et al. 2009), have garnered increasing attention for their usefulness as sewage indicators. PPCP study from continental (Kolpin et al. 2002; Focazio et al. 2008; Yang et al. 2018) to pore (Yang et al. 2016) scales, have shown that PPCP concentrations tend to be greatest closer to their source. In addition to identifying areas and periods of sewage pollution, PPCPs have also demonstrated robustness in defining gradients of sewage pollution in river systems, with concentrations being directly proportional to population density and inversely proportional to distance from a densely populated area (Bendz et al. 2005). Similar to PPCPs, microplastics (plastic debris up to 5 mm in size) also have been used to detect sewage pollution (Li et al. 2018) along gradients of increasing human population density (Klein et al. 2015). They are typically very resistant to degradation (Barnes et al. 2009), providing a signal over a longer time frame than many PPCPs and nutrients in sewage. As a result of each pollutant’s consistent association with sewage, co-located PPCP and microplastic measurements can inform duration as well as spatial extent of sewage pollution within an ecosystem.

By consistently defining sewage gradients, sewage indicator concentrations can be correlated with changes in primary producer and consumer guilds. Increased filamentous algae abundance, for example, has been frequently observed in areas suspected of sewage pollution (Rosenberger et al. 2008; Hampton et al. 2011), likely due to benthic filamentous algae efficiently removing nutrients from the water column (Hadwen and Bunn 2005; Andersson and Brunberg 2006). With a changing resource base, grazing macroinvertebrate communities may likewise shift to include more detritivores or species that are capable of consuming filamentous algae (Rosenberger et al. 2008). In addition to the physical difficulty grazers may experience in consuming filamentous algae (Mazzella and Russo 1989), there also may be changes in nutritional quality, as filamentous algae tend to contain a different mixture of essential fatty acids (EFAs) in comparison to diatoms (Kelly and Scheibling 2012). In particular, the EFAs 18:3ω3 and 18:4ω3 are commonly associated with filamentous algae (Taipale et al. 2013), whereas 20:5ω3, and 22:6ω3 are more associated with diatoms (Taipale et al. 2013). All EFAs are critical to maintaining cellular membrane fluidity especially in cold environments (Nichols et al. 1993), yet longer chain EFAs are generally more beneficial. Additionally, EFAs are largely synthesized by primary producers, each species of which usually produces a consistent EFA signature (Taipale et al. 2013). Consumers, however, can acquire EFAs by grazing (Dalsgaard et al. 2003) or upgrading EFAs at their own energetic expense (Sargent and Falk-Petersen 1988; Dalsgaard et al. 2003). In either event, comparing consumer fatty acid compositions to potential resources can be used to infer how grazing patterns may have changed in tandem with increasing sewage pollution.

To investigate community responses to sewage pollution, we surveyed 40 kilometers of Lake Baikal’s shoreline for indicators of sewage pollution and metrics of benthic community composition and structure. Located in Siberia, Lake Baikal is the oldest, most voluminous, and deepest freshwater lake in the world (Hampton et al. 2018), with the majority of Baikal’s biodiversity occurring in the littoral zone (Kozhova and Izmest’eva 1998). While Lake Baikal’s pelagic zone is generally ultra-oligotrophic (Ministry of Natural Resources and Ecology of the Russian Federation 2014), nearshore areas abutting lakeside settlements have shown distinct signs of eutrophication (Timoshkin et al. 2016). Much of Lake Baikal’s shoreline lacks human development and Baikal’s watershed being is largely roadless and unpopulated (Moore et al. 2009), yet uncharacteristic filamentous algal blooms have been occurring throughout the lake since 2010 (Kravtsova et al. 2014; Timoshkin et al. 2016; Volkova et al. 2018). While *Ulothrix* spp. blooms historically occur in late summer (Kozhov 1963; Kozhova and Izmest’eva 1998), *Spirogyra* spp. abundance is unprecedented and thought to be associated with increased nearshore nutrient concentrations (Volkova et al. 2018; Ozersky et al. 2018). Timoshkin et al. (2016, 2018) present a compelling case that inadequacies in wastewater management for lakeside settlements has been the main driver of nearshore algal blooms, motivating further research that might identify the extent to which sewage is altering nearshore communities.

Given the uncharacteristic changes in Baikal’s nearshore periphyton communities near lakeside settlements, the goal of our study was to understand how littoral benthic community composition and food web structure may be changing near areas with sewage pollution. Having evolved under ultra-oligotrophic conditions, Baikal’s biota present excellent opportunity to study how nearshore communities respond to nutrient addition. Even though Lake Baikal’s remoteness and high volume may mitigate risks of sewage pollution to the pelagic, nearshore biotic communities may be more susceptible to pollutants entering the lake from lakeside development. Additionally, Baikal’s diverse macroinvertebrates may each have varying responses to increasing sewage pollution, meaning that evaluating community compositions and interactions may detail nuanced responses. Consequently, our overarching goal can be divided into three main objectives:

1. to identify areas of sewage pollution using sewage-associated indicators,
2. to relate sewage indicators with changes in littoral benthic periphyton and macroinvertebrate community compositions, and
3. to evaluate how food webs may restructure with increasing sewage indicator concentrations.

We hypothesized that (1) sewage indicators, such as PPCP concentrations and microplastic densities, would increase with increasing population density and proximity of lakeside development; (2) an increasing sewage signal would correlate with increased dominance of filamentous benthic algae; and (3) increasing filamentous algae abundance will result in changes in the abundance of different feeding guilds, reflected in community composition as well as dietary tracers such as stable isotopes and fatty acids. In doing so, this study pairs highly specific sewage indicators to identify pollution hot spots with co-located biological measurements, thereby explicitly defining areas of sewage pollution.

**Methods**

1. *Site description*

The vast majority of Lake Baikal’s 2,000-km shoreline lacks lakeside development (Timoshkin et al. 2016). Our study focused on a 40-km transect of Baikal’s southwestern shoreline, which included three settlements of different size. We sampled 14 littoral and 3 pelagic locations along our 40-km transect. Littoral locations were chosen to capture a range of sites with varying degrees of adjacent shoreline development – from “developed” (no human settlements and complete natural forest cover) to “undeveloped” (along the built-up waterfront of Listvyanka). Pelagic sites were located 2 to 5 km offshore from each of the developed sites (Figure 1; Table 1). Littoral sites were sampled at the same depth (~1.25 m) and therefore the sampling locations sometimes differed in their distance from shore (Table 1). At each site, air temperature was measured with a mercury thermometer and photographs were taken of the substrate and the shoreline.

Along our 40-km transect, there were three discrete lakeside settlements. The largest, Listvyanka, is primarily a tourist town with approximately 1,963 permanent residents, although tourism can contribute significantly to the town’s population with 1.2 million visitors over the course of the year (IrkutskStat, 2012, Timoshkin et al., 2016). The other two settlements are the villages Bolshie Koty and Bol’shoe Goloustnoe have approximately 80 and 600 permanent residents, respectively. Bolshie Koty is home to two field research stations and several small hotels. Bolshoe Goloustnoe largely hosts tourist operations (IrkutskStat, 2012).

*Inverse distance weighted (IDW) population calculation*

At each littoral sampling location, we recognized that sewage indicator concentrations may be related to a sampling location’s position relative to the size and proximity of neighboring lakeside developments. We, therefore, created the inverse distance weighted (IDW) population metric, which reflects the idea that sewage pollution should be positively related to increasing human density and inversely related with distance from densely populated areas (sensu Bendz et al., 2005). Additionally, Timoshkin et al. (2018) noted that sewage into Baikal’s nearshore largely enters through subsurface-surface connections, implying that locations with more direct shoreline development should experience higher sewage concentrations in the lake as PPCPs (Karnjanapiboonwong et al., 2010) and nutrients (de Vries, 1972) can be removed via the soil matrix. Acknowledging that nearshore PPCP concentrations were likely positively proportional to a developed location’s shoreline length, we scale a developed sites population density by its shoreline length.

Our workflow for calculating IDW population can be described in five main steps. First, we traced polygons and shoreline lines from satellite imagery for each development site within Google Earth (citation??). Second, polygon and line geometries were downloaded locally as a .kml file. Third, the .kml file was imported into the R statistical environment (R Core Team, 2019), where using the sf package (Pebesma, 2018) we calculated shoreline length, polygon area, and centroid location for each developed site. Fourth, we then joined point locations for each sampling site with the spatial polygons so as to calculate the distance from each sampling location to each development’s centroid. Fifth, we used the dplyr package to calculate IDW population for each location, using the formula , where Ij is the IDW population at site j, P is the population at a developed site, A is the area of a developed site in km2, L is the shoreline length at a developed site km, and D is the distance from developed site j to each developed site in km. This equation implies that all sampling locations are influenced by all three developed sites; the relative influence of an individual site is inversely proportional to a sampling location’s distance from each of the three developed sites.

*2. Water samples*

At both pelagic and littoral sites, water samples were collected for nutrient, chlorophyll, microplastic, and pharmaceutical and personal care product (PPCP) analysis. Each water sample collection procedure is described below.

*2a. Nutrients*

Water samples for nutrient analyses were collected in approximately 150 mL glass jars that had been rinsed three times with water from the sampling location. Samples were collected in duplicates and immediately frozen at -20°C until processing at the Siberian Branch of the Russian Academy of Sciences.

For each water sample, nitrate, ammonium, phosphate, and total phosphorus concentrations were measured. For ammonium and nitrate concentrations, samples were analyzed with a spectrophotometer following the addition of Nessler’s reagent and disulfuric acid respectively. Phosphate concentration was measured with a spectrophotometer following the addition of persulfate. Concentrations are reported in mg/L.

*2b. Chlorophyll a*

Water samples were collected in 1.5 L plastic bottles from a depth of approximately 0.75 m. Within 12 h of collection, three subsamples were collected by passing up to 150 mL through 25 mm cellulose nitrate filter. Filters were then placed in a 35 mm petri dish and frozen in the dark until processing.

Chlorophyll samples were processed in a manner similar to that of Parson (1963) and Lorenzen (1967). Filtered GF/Fs were ground in 90% acetone, in which chlorophyll extraction was allowed to proceed overnight. Samples were centrifuged for 15-20 minutes. Once particulates settled, absorbance of the chlorophyll extract was measured in a spectrophotometer at 630, 645, 665, and 750 nm. Concentrations were calculated using the formula: ; where A is the absorbance value of a particular wavelength, V1 is the volume of the filtered water, and V2 is the volume of extract. Concentrations are reported as mg/L.

*2c. PPCPs*

Water samples were collected in 250 mL amber glass bottles that were rinsed with either methanol or acetone and then three times with sample water prior to collections. Following collection, samples were refrigerated and kept in the dark until solid phase extraction (SPE).

Within 12 h of collection, samples were filtered directly from the amber glass bottle using a 25 mm GF/F, and then passed through SPE cartridges (Waters Corporation, Milford, MA). Lab personnel wore gloves and face masks to minimize contamination. Prior to filtration, SPE cartridges were primed with at least 5 mL of either methanol or acetone and then washed with at least 5 mL of sample water. Rate of SPE occurred at approximately 1 drop per second. Extraction proceeded until water could no longer pass through the SPE cartridge or until all collected water was filtered. Cartridges were stored in whirlpacks at -20C until analysis for PPCPs following methods of Lee et al. (2016).

2d. *Microplastics*

At each location, samples were collected in triplicate using 1.5 L clear plastic bottles that were washed thoroughly with sample water before each collection. Samples were collected by hand from the midpoint depth for each littoral site and with a bucket from aboard the ship for pelagic sites.

For processing, each sample was vacuum filtered on to a 47 mm GF/F filter. During filtration, aluminum foil was used to cover the filtration funnel to prevent contamination. Upon completion, filters were allowed to dry under vacuum pressure and then stored in 50 mm petri dishes. Following filtration of all three replicates, filtrate was collected and then re-filtered as a control for contamination.

Microplastic counting involved visual inspection of the entire GF/F according to guidelines proposed in Van Cauwenberghe et al. (2015). Visual enumeration was conducted under a stereoscope, and microplastics were classified into one of three categories: fibers, fragments, or beads. For all categories, plastics were defined as observed objects with apparently artificial colors. Fibers were defined as smooth, long plastics with consistent diameters. Fragments were defined as plastics with irregularly sharp or jagged edges. Beads were defined as spherical plastics. During enumeration, GF/Fs remained in the petri dish to minimize potential for contamination from the air. Following enumeration of both experimental and control samples, fibers, fragments, and beads enumerated in the controls were subtracted from the experimental microplastic densities for each plastic type and from each replicate. One location (BK-1) had two control replicates, which were averaged for each plastic type and then subtracted from the experimental samples. Results are reported as the average number of microplastics per liter.

*3. Benthic biological samples*

At each littoral site, periphyton and macroinvertebrates were collected for relative abundance estimates and food web analysis.

*3a. Benthic algal collection*

At each littoral site, we haphazardly selected three rocks representative of local substrate. A plastic stencil was used to scrape a standardized 14.5 cm2 patch of periphyton from the upper surface of each rock. Samples were stored in plastic scintillation vials and preserved with Lugol’s solution. Remaining periphyton from the site were collected in composite for fatty acid and stable isotope analysis to enable food web characterization (described below).

Periphyton taxonomic identification and enumeration was performed by subsampling 10 μL aliquots from each preserved sample. For the entire aliquot, cells, filaments, and colonies were counted for each taxonomic group until at least 300 cells were identified. In instances where 300 cells were counted prior to completing the aliquot, the entire aliquot was still counted. Taxa were classified in broad categories consistent with Baikal algal taxonomy (Izhboldina, 2007), using coarse groupings so as to capture overarching patterns in relative algal abundance. As a result, algal groups consisted of diatoms (as a whole group), *Ulothrix*, *Spirogyra*,and Tetrasporales.

*3b. Benthic invertebrate collection*

At each littoral site, three kick-net samples were collected for assessment of benthic community composition and abundance. Using a D-net, we collected macroinvertebrates by flipping over 1-3 rocks, and then sweeping five times in a left-to-right motion for approximately 1 m. After the series of sweeps, the catch was rinsed into a plastic bucket. For each replicate, bucket contents were concentrated using a 64 μm net and placed in glass jars with 40% ethanol (vodka; which was the only preservative available to us at the time) for preservation and refrigerated at 4°C aboard the research vessel. The 40% ethanol preservative was replaced with ~80% ethanol upon return to the lab within 24-48 hours, and samples were stored at ~4°C.

Separate collections were conducted for invertebrate fatty acid and stable isotope analyses. Invertebrates were collected using a D-net in a similar fashion as the community enumeration. Additional invertebrates were also collected by hand. Collected organisms were then live-sorted, identified to species, and frozen at -80°C.

Invertebrate taxonomic identification and enumeration were performed under a stereoscope. All well preserved invertebrates were identified to species with the exception of juveniles (Taakhteev, 2015 for amphipods; Sitnikova, 2012 for molluscs; Table 2). Samples that were not well preserved were not counted so as to prevent erroneous abundances. To create a characteristic invertebrate taxonomic count, we averaged invertebrate counts for each site.

*3c. Food web characterization*

To characterize littoral food webs, we analyzed carbon and nitrogen stables isotopes as well as fatty acid profiles for periphyton and macroinvertebrates. Prior to isotopic and fatty acid analysis, periphyton and macroinvertebrate samples were freeze dried for ~48 hours, homogenized to powder, and then massed.

*Stable isotope analysis*

Measurements of δ15N and δ13C were performed on an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS; Finnigan DELTAplus XP, Thermo Scientific). The EA-IRMS was calibrated against certified reference materials including L-glutamic acid (NIST SRM 8574), low organic soil and sorghum flour (standards B-2153 and B-2159 from Elemental Micro-analysis Ltd., Okehampton, UK) and in-house standards (acetanilide and caffeine).

*Fatty acid analysis*

Fatty acid extractions generally involved three phases: (1) 100% chloroform extraction, (2) chloroform-methanol extraction, and (3) fatty acid methylation. Fatty acid extraction methods were adapted from similar methods developed in Schram et al. (2018).

Samples were first freeze-dried in Eppendorf tubes at -20°C overnight, then ground and weighed. Following freeze-drying, samples were transferred to 10 mL glass centrifuge vials, and 2 mL of 100% chloroform was added to each under nitrogen gas. Samples were allowed to sit in chloroform overnight at -80°C.

Following overnight chloroform extraction, samples underwent a chloroform-methanol extraction three times. To each sample, we added 1 mL cooled 100% methanol, 1 mL chloroform:methanol solution (2:1), and 0.8 mL 0.9% NaCl solution. Samples were inverted three times and sonicated on ice for 10 minutes. Next, samples were vortexed for 1 minute, and centrifuged for 5 minutes (3,000 rpm) at 4°C. Using a double pipette technique, the lower organic layer was extracted and kept under nitrogen. After the third extraction, samples were allowed to evaporate under nitrogen flow, and resuspended in 1.5 mL chloroform and stored at -20°C overnight.

Once resuspended in chloroform, 1 mL of chloroform extract was transferred to a glass centrifuge tube with a glass syringe. As a standard, 4 μL of 19-carbon fatty acid was added along with 1 mL of toluene and 2 mL of 1% sulfuric acid-methanol. The vial was closed under nitrogen gas and then incubated in 50°C water bath for 16 hours. After incubation, samples were removed from the bath, allowed to reach room temperature and stored on ice. Next, we performed a potassium carbonate-hexane extraction twice. To each sample, we added 2 mL of 2% potassium bicarbonate and 5 mL of 100% hexane, inverting the capped vial so as to mix the solution. Samples were centrifuged for 3 minutes (1,500 rpm) at 4°C. The upper hexane layer was then removed and placed in a vial to evaporate under nitrogen flow. Once almost evaporated, 1 mL of 100% hexane was added and stored in a glass amber autosampler vial for GC/MS quantification. GC/MS quantification was performed with a Shimadzu QP2020 GC/MS in a similar method as described in Schram et al. (2018).

*4. Statistical analyses*

Total phosphorus, nitrate, ammonium, microplastic abundance and density, total PPCP concentration, and δ15N in macroinvertebrate tissues were log-transformed and regressed against log-transformed IDW population using a linear model. Analytically, log-transforming made sites with a wide range of values more comparable to one another. Physically, we assumed that sewage indicators were likely subject to exponential processes (e.g., mixing, diffusion), and log-transforming the data should linearize the relationships between predictor and response variables. Residuals were assessed for normality and homogeneity of variance.

To assess if benthic community composition was associated with increasing sewage indicators, periphyton and macroinvertebrate abundance data were each analyzed with a consistent multivariate routine. First, taxonomic groups representing less than 1% of the inter-site community were removed from analysis, so as to potentially remove the influence of rare species. Second, community compositions for both periphyton and macroinvertebrates were visualized using non-metric multidimensional scaling (NMDS) with a Bray-Curtis similarity metric. Periphyton community compositions were calculated as relative proportions, whereas invertebrate abundances were square-root transformed to minimize influence of more abudant taxa. Visual inspection of the NMDS plot suggested that sites tended to cluster by low, moderate, and high PPCP concentrations and IDW population. Third, we used k-means clustering to identify an optimal number of clusters (Figure S1). This method allowed us to determine if two or three clusters could be used to describe the data, where two clusters consisted of low/moderate PPCP concentrations or IDW population and three consisted of low, moderate, and high PPCP concentrations or IDW population. We then iterated through multiple numbers of clusters (i.e., 1 to 10) and calculated the within-group-sum-of-squares (WSS). We identified the optimal number of clusters when WSS decreased most markedly. To assess whether differences between groups were significant, we performed a permutational multivariate analysis of variance (PERMANOVA, (Anderson 2001)) with 999 permutations, where community compositions were treated as a response to the groups identified through k-means clustering. Unlike traditional multivariate analyses of variance (MANOVA), PERMANOVA does not require assumptions of multivariate normality (Anderson 2001). Post-hoc SIMPER analysis (Clarke 1993) was performed following the PERMANOVA to identify which taxonomic groups most influenced cluster differences.

To assess if benthic food webs restructured with increasing sewage indicator concentrations, fatty acid data were analyzed in a manner similar to periphyton and macroinvertebrate abundance data. First, species’ fatty acid profiles were visualized by performing NMDS with Bray-Curtis similarity for all organisms (Figure S2). This technique broadly demonstrated that inter-specific variation in fatty acid composition was greater than intraspecific variation. The same pattern was observed for all fatty acids quantified as well as solely essential fatty acids (EFAs; Figure S2). The NMDS plot with species’ EFA profiles suggested that sites suggested visually that sites discriminated based on sewage indicator concentrations. Among the eight common EFAs used with an ecological focus (Taipale et al. 2013), 18:3ω3, 18:4ω3, 20:5ω3, and 22:6ω3 had the highest coefficient of variation between sites, and so we focused our analysis on these four compounds. Additionally, these four EFAs are also indicative of Green algae (i.e., 18:3ω3 and 18:4ω3) as well as diatoms (i.e., 20:5ω3 and 22:6ω3). To evaluate how relative EFA abundance may be related with sewage pollution, we regressed filamentous:diatom fatty acid signals (i.e., (18:3ω3% + 18:4ω3%)/(20:5ω3% + 22:6ω3%)) against log-transformed PPCP concentrations using a linear model.

All analyses were conducted within the R statistical environment (R Core Team 2019), using the tidyr (Wickham and Henry 2019), dplyr (Wickham et al. 2019), ggplot2 (Wickham 2016), and vegan (Oksanen et al. 2019) packages. All data are publicly available from the Dryad data repository (DOI), and all R scripts are available from the GitHub repository of this project’s Open Science Framework account (DOI).

**Results**

*1. Water samples*

Nitrate (R2 = 0.01, p = 0.62), ammonium (R2 = 0.15, p = 0.12), and chlorophyll a (R2 = 0.11, p = 0.20) were not significantly correlated with IDW population (Figure 2). Phosphate (R2 = 0.20, p = 0.07) approached significance, and total PPCP (R2 = 0.31, p = 0.022) concentrations, were significantly related with IDW population (Figure 2). Within the littoral zone, detected PPCPs included caffeine, 1,7-dimethylxanthine (main human metabolite of caffeine), cotinine (main human metabolite of nicotine), and acetaminophen (Table 3).

Microplastics were detected in samples from both the littoral and pelagic sites. Bead microplastics were only detected at sites in Listvyanka. Fibers (mean = 0.85 microplastics/L, std dev = 1.21 microplastics/L) and fragments (mean = 0.83 microplastics/L, std dev = 1.35 microplastics/L) were the most abundant types of microplastics across all sites, whereas beads were relatively rare beads (mean = 0.08 microplastics/L, std dev = 0.31 microplastics/L). Total microplastic densities were not significantly correlated with IDW population (R2 = 0.03, p = 0.53; Figure 2), although more types of microplastics were generally observed near areas with higher IDW population values, such as Listvyanka.

*2. Benthic biological samples*

*2a. Periphyton*

Major taxonomic groupings of periphyton consisted of diatoms, *Tetrasporales* spp*.*, *Spirogyra* spp., and *Ulothrix* spp. K-means cluster analysis of periphyton abundance demonstrated three groupings capture most variance, and visual inspection of periphyton community NMDS suggested groupings were related to IDW population values (Figure 3). PERMANOVA results demonstrated that periphyton communities were significantly different based on IDW populations (R2 = 0.55, p = 0.023). Post-hoc SIMPER results suggested sites with high IDW populations as having higher *Ulothrix* spp. relative abundance in comparison to moderate and low IDW populations. Additionally, sites with high IDW populations had higher diatom relative abundance in comparison to sites with low IDW populations, yet not for sites with moderate IDW populations.

*2b. Macroinvertebrates*

Taxonomic groupings included four amphipod genera: *Eulimnogammarus*, *Poekilogammarus*, *Cryptoropus*, *Brandtia and* *Pallasea*; two mollusc families: Planorbidae, Valvatidae, Baicaliidae, Benedictidae, Acroloxidae, Maackia; flatworms; caddisflies; and leeches (summarized in Table 2). K-means cluster analysis of macroinvertebrate community composition demonstrated 3 major groupings would capture most variance, and visual inspection of NMDS suggested clusters were related to IDW population (Figure 4). PERMANOVA results supported the hypothesis that macroinvertebrate communities significantly differed along a gradient of IDW populations (p = 0.001). Post-hoc SIMPER analyses suggested *Poekilogammarus* abundance was significantly different between IDW population groupings. Nevertheless, *Poekilogammarus*, *Eulimnogammarus*, *Valvatidae*, Caddisflies, *Brandtia*, *Baicaliidae*, and *Panorbidae* contributed the greatest differences between High and Moderate/Low IDW population groupings.

*3. Food web characterization: stable isotopes and fatty acids*

Among periphyton and amphipod samples, δ 13C values ranged from -19.5 to -9.5% (Figure 5). Among grazer δ15N values, no grazer groups deviated greater than 3.4% δ15N than the lowest grazer δ15N values, suggesting that all were within the same trophic level (Post 2002).

δ15N significantly increased with IDW population only for grazers (p = 0.008; Figure 2, Figure 5). Periphyton δ15N signatures did not significantly increase with IDW population (p = 0.7). In contrast, δ 13C concentrations were not related with IDW population for either periphyton or macroinvertebrates.

For both periphyton and grazers, our analyses focused mainly on the essential fatty acids (EFAs) 18:3ω3, 18:4ω3, 20:5ω3, and 22:6ω3. For periphyton, the ratio of C18:3ω3 and C18:4ω3 in comparison to C20:5ω3 and C22:6ω3 significantly increased with an increasing PPCP concentration (p = 0.05, Figure 6) but not with an increasing IDW population (p = 0.17). Amphipods’ fatty acid ratios were not significantly related with either increasing IDW population or increasing PPCP concentrations.

**Discussion**

Our combined results corroborate previous findings (e.g., Timoshkin et al., 2016; 2018) that sewage pollution is entering Lake Baikal’s nearshore area and likely altering nearshore benthic communities. Unlike previous studies, though, we incorporate highly nuanced indicators of sewage pollution and food web structure to offer a holistic perspective to how localized development may affect nearshore benthic communities.

*Relating human settlements to sewage indicator concentrations*

Concordant with our expectations, sewage pollution indicators in the nearshore of Lake Baikal were associated with size of and distance from human settlements. Total PPCP, macroinvertebrate δ15N, and to some degree total phosphorus concentrations increased with IDW population. These gradients from highly localized settlements are noteworthy considering that Baikal’s shoreline, including our study area, is largely void of lakeside development (Moore et al. 2009). Furthermore, the use of consistently sewage-associated micropollutants, such as PPCPs and δ15N proved necessary for defining sewage gradients. Despite Lake Baikal lacking major human population centers, melting permafrost within Lake Baikal’s (Anisimov & Reneva, 2006) and the Selenga (Tornqvist et al., 2014) have the potential to contribute substantial nutrient loadings. Callender & Granina (1997) demonstrated that up to 70% of Lake Baikal’s phosphorus loading historically comes from geological sources. Melting permafrost, especially in the Southern basin, is predicted to be severe and of high risk for infrastructure (see Figure 5 in Anisimov & Reneva, 2006). In turn, the potential for melting permafrost to contribute to localized eutrophication heightens the need for sewage-specific indicators, like PPCPs, such that monitoring efforts can identify sources of nutrient pollution.

To the authors’ knowledge, this study is the first to detect PPCPs within Lake Baikal. While PPCPs have been found in numerous aquatic systems (Kolpin et al. 2002; Focazio et al. 2008; Rosi et al. 2013), lakes have remained less represented within the PPCP literature in comparison to lotic and subsurface systems (Meyer et al. 2019). With longer hydraulic residence times, PPCP distributions in lentic systems may differ from lotic environments, as pollutants within lakes may be more prone to accumulate within the nearshore before diffusing to undetectable concentrations offshore. Our results support this idea, showing patchy concentrations of PPCPs nearshore and undetectable concentrations at the offshore sites. We do not, however, address how the PPCPs themselves may influence community dynamics. Previous studies have shown that PPCPs, even in minute concentrations (e.g., ng/L) can elicit biological responses from physiological (e.g., Del Rosario et al., 2015) and behavioral (e.g., Brodin et al. 2013; Dzieweczynski et al., 2016) to food web (e.g., Lagesson et al., 2016; Richmond et al., 2018) and ecosystem (e.g., Rosi-Marshal et al., 2013). Although our study does not address effects of PPCPs themselves, our data do demonstrate that multiple PPCPs can concentrate within Baikal’s nearshore area to a level where they may elicit their own effects.

In contrast to PPCP and δ15N concentrations, microplastics at our sampling sites were not associated with IDW population and may be a poor proxy for sewage pollution in Lake Baikal. Microplastics have been used to identify sewage pollution in other large lakes, like Lake Hovsgol (Free et al. 2014), Lake Superior (Hendrickson et al., 2018), and Lake Erie (Eriksen et al., 2013). Unlike PPCPs’, which have relatively short half-lives, microplastics indicate accumulated pollution because of their slow degradation time (Brandon et al. 2016), which likely promotes wider distribution from nearshore inputs to the offshore (Hendrickson et al., 2018; Fischer et al., 2016). Microplastic concentrations in Baikal, as quantified by our methods, may be poor proxies for capturing pollution from seasonally varying human populations, but may be useful in detecting offshore sewage signals as well as in calculating a time-integrated sewage signal. Additionally, microplastics carry the caveat that plastics may originate from non-sewage sources, such as agriculture (Steinmetz et al., 2016). It is worth noting that since the time of our field sampling, evidence has accumulated that our methods likely dramatically underestimated microplastic abundance (Wang and Wang 2018; Brandon et al. 2020). While we focus here on microplastics as an indicator of human disturbance, microplastics are increasingly shown to disrupt food web dynamics by altering grazing patterns (Green 2016) and providing carbon substrate for microbial growth (Romera-Castillo et al. 2018). Together these growing uncertainties suggest that microplastic pollution in Baikal and elsewhere deserves increased attention.

*Relating sewage indicators with benthic algal communities*

Congruent with our hypotheses, increasing sewage indicators tended to be associated with more filamentous periphyton. Previous studies investigating Baikal’s periphyton composition noted that areas adjacent to human development often had increased abundance of filamentous algae such as Ulothrix and Spirogyra (Timoshkin et al. 2016, 2018). Lake Baikal’s southwestern shore historically experiences short *Ulothrix* blooms in late August (Kozhov 1963), potentially confounding sewage signals with an annually occurring phenomenon. Our data agree with results of Timoshkin et al. (2016) and show that relative abundance of filamentous algae is greatest near areas of high lakeside development. This changing periphyton community composition implies changing nutritional quality. Periphyton essential fatty acid profiles from sites with higher sewage pollution had higher levels of 18:3ω3 and 18:4ω3 relative to C20:5ω3 and C22:6ω3 fatty acids. Because fatty acid profiles tend to be indicative of algal species (Iverson et al. 2004; Taipale et al. 2013; Galloway and Winder 2015), this pattern likely reflects the higher abundance of green algae relative to diatoms. (Osipova et al. 2009; Taipale et al. 2013). Stable isotopes, however, suggested no differences in periphyton δ15N with increasing IDW population (Figure 5). While increased δ15N concentrations can be indicative of sewage pollution (Tucker et al., 1999), periphyton’s typically high cell turnover rates likely dampens isotopic patterns (e.g., days; (Swamikannu and Hoagland 1989)). Together, these results suggest that Baikal’s nearshore periphyton communities near human lakeside developments are becoming more dominated by filamentous algae, and therefore, likely changing their nutritional content.

Among the array of fatty acids synthesized in algal communities, essential fatty acids (EFAs) are most likely to be taxonomically associated and influenced by changing community compositions. EFAs are a subgroup of polyunsaturated fatty acids (PUFAs) that are prone to accumulating within organisms (see Kelly & Scheibling, 2012). Among the eight common EFAs used with an ecological focus (Taipale et al. 2013), 18:3ω3, 18:4ω3, 20:5ω3, and 22:6ω3 had the highest coefficient of variation between sites. Because these four EFAs demonstrated the greatest variation between sites, our analyses focused on how their relative abundances related to PPCP concentrations and IDW populations. The fatty acids 18:3ω3 and 18:4ω3 have been previously associated with filamentous algae, such as Baikalian *Ulothrix* (Osipova et al. 2009), whereas 20:5ω3 and 22:6ω3 have previously been associated with diatoms (Taipale et al. 2013). Comparing the ratio of (18:3ω3% + 18:4ω3%)/(20:5ω3% + 22:6ω3%) could therefore function as proxy for filamentous:diatom abundance and potentially offer insights into feeding patterns for the grazers.

*Relating sewage indicators with macroinvertebrate feeding guilds*

With respect to how benthic consumer communities would restructure with a changing periphyton community and increasing sewage signal, our data suggest a nuanced macorinvertebrate grazer response. In contrast to Timoshkin et al.’s (2016) reported mass mortality of snails, our results also support those authors’ general conclusion that Baikalian mollusc abundance tends to decrease within increasing sewage pollution. Decreased mollusc abundance may have several causes, including molluscs having low tolerance for increased PPCP concentrations (e.g., Hollingsworth et al. 2002), molluscs not being able to consume filamentous algae (Mazzella and Russo 1989), filamentous algae not offering the proper nutritional yield for molluscs (Lowe and Hunter 1988), or certain contaminants within the sewage being particularly toxic to molluscs (Timoshkin et al. 2016). In contrast to molluscs, amphipods were generally prevalent at all littoral sites regardless of sewage indicator concentrations. *Brandtia* spp. was the only species less prevalent with sewage indicator signals; this genus, however, tends to be associated with endemic sponges (Taakhteev & Didorenko, 2015), which may also be decreasing in abundance near areas of lakeside development (Timoshkin et al., 2016). With respect to amphipod nutritional quality, amphipod fatty acid profiles did not differ markedly between sites (Figure 6), whereas δ15N increased slightly but significantly with increasing IDW population. The differing pattern of periphyton and amphipod δ15N values in relation to IDW population may relate to amphipods having longer cellular turnover rates (e.g., weeks; McIntyre and Flecker 2006) relative to periphyton. These combined results suggest that mollusc abundance and amphipod δ15N concentrations may be longer-term indicators of sewage pollution in Baikal.

While amphipod fatty acid profiles were not associated sewage signals, amphipods from all collected sites expressed consistent 20:5ω3 and 22:5ω3 signatures relative to 18:3ω3 and 18:4ω3. Unlike primary producers, consumers usually accumulate fatty acids from their food source. Yoshii’s (1999) study use of stable isotope data suggested that Baikal’s littoral amphipods likely graze on benthic algae. Because fatty acid profiles in amphipods largely reflect fatty acid signatures in periphyton, our data suggest that amphipods likely continue grazing on periphyton, despite the food resource changing in community composition and nutritional content. As a consequence, amphipods may be compensating for periphyton’s shifting nutritional status through a minimum of two potential mechanisms. First, amphipods may selectively consume diatoms as opposed to filamentous algae, meaning diatom relative abundance could decrease both from increased grazing and lesser efficiency at removing nutrients relative to filamentous taxa. Second, amphipods may indeed consume filamentous algae, and then invest energy to convert C18 fatty acids to C20 and C22 fatty acids. Regardless of the exact mechanism though, our data suggest that food web interactions would alter with increasing sewage pollution and imply a net energetic cost through amphipods’ differential grazing patterns.

*Conclusions*

Over the past decade, Lake Baikal has shown signs of nearshore eutrophication throughout the lake’s entirety, despite the pelagic remaining ultra-oligotrophic. While Baikal may have multiple sources of nutrient inputs, including melting permafrost, our results corroborate previous studies by showing sewage pollution as one of the potentially numerous sources. Adding to foundations lain by Timoshkin et al. (2016, 2018) and Ozersky et al. (2018), our results demonstrate how patchy hot spots of lakeside development can create gradients in sewage concentrations and ecological responses. Unlike previous studies, our study pairs co-located measurements of sewage pollution (i.e., PPCPs) with nuanced dietary tracers (i.e., fatty acids) to assess benthic community and food web consequences of sewage pollution. In general, our results suggest that while sewage pollution may lead to changing standing resources for macroinvertebrate grazers, Baikal’s amphipods appear to be compensating either by selectively grazing on diatoms or by consuming less desirable food and upgrading fatty acids. In both cases, our results suggest shifting community interactions and imply a net energetic cost for amphipods, as they expend energy either by scavenging explicitly for diatom or by catabolizing fatty acids necessary to survive in Baikal’s cold temperatures.

*Future trajectories: a call for increased nearshore monitoring*

With respect to future trajectories, our results underscore the importance of nearshore monitoring in detecting sewage pollution. In the case of Baikal, the Ministry of Natural Resources and Ecology of the Russian Federation (2014) report was based on pelagic samples and deemed Baikal to be ultra-oligotrophic, while eutrophic hot spots developing throughout the lake suggest localized eutrophication. While pelagic samples may be more representative of the lake’s overall integrity, nearshore sampling aids managers to pinpoint pollution loading before the entire system is affected (Hampton et al., 2011). Beyond Baikal, several large, deep, oligotrophic lakes have likewise experienced localized sewage pollution with nearshore biological responses, despite the pelagic suggesting oligotrophic states (e.g., Rosenberger et al. 2008; Hampton et al., 2011). This discrepancy between monitoring programs’ focus on pelagic sampling despite nearshore responses is noteworthy, if the goal of monitoring programs is to signal trophic status changes before they occur. Because systems may experience low level sewage inputs or even nutrient inputs from multiple sources, incorporating sewage specific indicators, such as PPCPs, may be necessary, especially as lakes have historically been underrepresented in the PPCP literature (Meyer et al., 2019). Our study contributes to the body of nearshore sampling work by detailing that even when lakeside development may occur in isolated zones, gradients can form within the nearshore area and propagate community and food web responses even when the human disturbance is not adjacent to the ecological communities.

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**Acknowledgments**

We would like to thank the faculty, students, staff, and mariners of the Irkutsk State University’s Biological Research Institute Biostation for their expert field, taxonomic, and laboratory support; Marianne Moore and Bart De Stasio for helpful advice; the researchers and students of the Siberian Branch of the Russian Academy of Sciences Limnological Institute for expert taxonomic and logistical assistance; Stephen M. Powers, Stephanie G. Labou, Stephen L. Katz, Brian P. Lanouette, John R. Loffredo, Alex K. Fremier, Erica J. Crespi, Daniel L. Preston, and Jim J. Elser for offering insights throughout the development of this project. Funding was

provided by the National Science Foundation (NSF-DEB-1136637) to S.E.H., a Fulbright Fellowship to M.F.M., a NSF Graduate Research Fellowship to M.F.M. (NSF-DGE-1347973), and the Russian Ministry of Education and Science Research Project (No. GR 01201461929; 1354-2014/51). This work serves as one chapter of M.F.M.’s doctoral dissertation in Environmental and Natural Resource Sciences at Washington State University.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Site | Latitude | Longitude | Depth (m) | Distance to shore (m) | Air Temperature (C) | Surface Temperature (C) | Adjacent Population |
| BK-1 | 51.90316 | 105.07404 | 0.7 | 10 | 18 | 14 | 56 |
| BK-2 | 51.90365 | 105.069 | 0.9 | 17.5 | 19 | 13 | 56 |
| BK-3 | 51.90536 | 105.0957 | 0.8 | 10 | 18 | 14 | 56 |
| BGO-1 | 52.02693 | 105.40102 | 0.9 | 18 | 20 | 13 | 0 |
| BGO-2 | 52.0197 | 105.37707 | 1.1 | 14 | 19 | 14 | 600 |
| BGO-3 | 52.02649 | 105.43577 | 0.7 | 21 | 18 | 16 | 600 |
| OS-1 | 51.98559 | 105.47237 | 900 | NA | 15 | NA | NA |
| KD-1 | 51.92646 | 105.24504 | 0.8 | 20.75 | 23 | NA | 0 |
| KD-2 | 51.91807 | 105.21456 | 0.9 | 14.5 | 23 | 16 | 0 |
| MS-1 | 51.89863 | 105.15017 | 0.6 | 10.5 | 21 | 17 | 0 |
| SM-1 | 51.87152 | 104.98006 | 0.9 | 11.5 | 21 | 15 | 0 |
| LI-1 | 51.86825 | 104.83042 | 0.6 | 8.9 | 19 | 14 | 2000 |
| LI-2 | 51.84626 | 104.87356 | 0.8 | 9.4 | 21 | 15 | 2000 |
| LI-3 | 51.85407 | 104.86216 | 0.7 | 9.25 | 19.5 | 15 | 2000 |
| EM-1 | 51.86005 | 104.93999 | 0.7 | 15.5 | 24.5 | 14 | 0 |
| OS-2 | 51.8553 | 104.8148 | 1300 | NA | 21 | NA | NA |
| OS-3 | 51.859108 | 105.0769 | 1400 | 5000 | NA | 14.5 | NA |

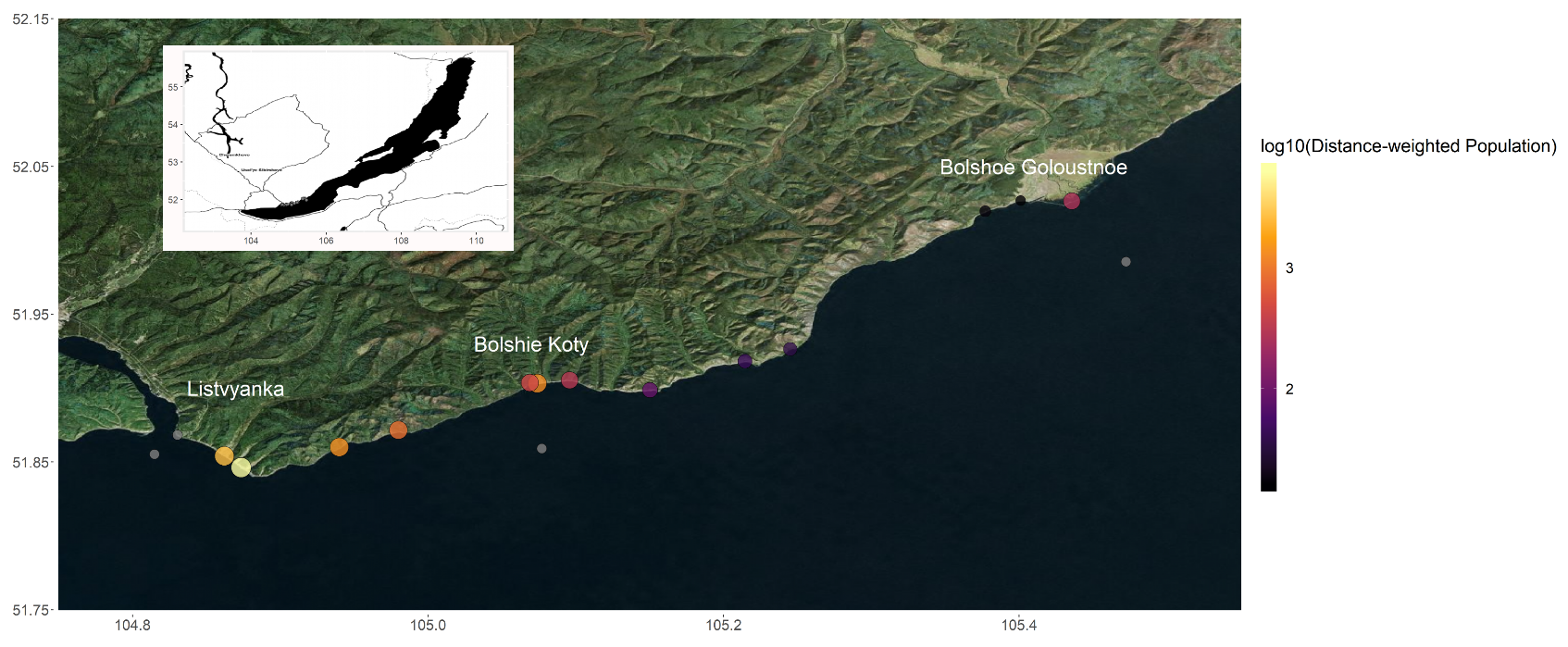


Figure 1: Map of all sampling locations with sites sized and colored by IDW population. Grey sites are located where a IDW population could not be calculated. The entire transect included three developed sites (i.e., Listvyanka, Bolshie Koty, Bolshoe Goloustnoe). Listvyanka contains approximately 2,000 permanent residents, although it experiences a transient tourist population of approximately 300,000 people annually. Bolshie Koty contains approximately 56 permanent residents, but the two local field stations and tourism can increase population by 200+ individuals in the summer. Bolshoe Goloustnoe has approximately 600 permanent residents and remains relatively consistent throughout the year. Three offshore samples were also collected to compare pelagic sewage signals to those in the littoral.

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| Table 2: Macroinvertebrate taxonomic groupings for abundance estimates. Amphipod taxa were defined as in Taakhteev & Didorenko, 2015; Mollusc taxa were defined as in Sitnikova, 2012. | | |
| **Amphipoda** | **Mollusca** | **Other** |
| *Brandtia latissima intermida* | Acroloxidae | Asellidae |
| *Brandtia latissima lata* | Baicaliidae | Caddisflies |
| *Brandtia latissima latior* | Benedictidate | Hirudinea |
| *Brandtia latissima latissima* | Maackia | Planaria |
| *Brandtia latissima lora* | Planorbidae |  |
| *Brandtia parasitica parasitica* | Valvatidae |  |
| *Cryptoropus inflatus* |  |  |
| *Cryptoropus pachytus* |  |  |
| *Cryptoropus rugosus* |  |  |
| *Eulimnogammarus capreolus* |  |  |
| *Eulimnogammarus cruentes* |  |  |
| *Eulimnogammarus cyaneus* |  |  |
| *Eulimnogammarus grandimanus* |  |  |
| *Eulimnogammarus maacki* |  |  |
| *Eulimnogammarus marituji* |  |  |
| *Eulimnogammarus verucossus* |  |  |
| *Eulimnogammarus viridis viridis* |  |  |
| *Eulimnogammarus vittatus* |  |  |
| *Hyallela cziarnianski* |  |  |
| *Pallasea brandtia brandita* |  |  |
| *Pallasea brandtii tenera* |  |  |
| *Pallasea cancelloides* |  |  |
| *Pallasea cancellus* |  |  |
| *Pallasea viridis* |  |  |
| *Poekilogammarus crassimus* |  |  |
| *Poekilogammarus ephippiatus* |  |  |
| *Poekilogammarus megonychus perpolitus* |  |  |
| *Poekilogammarus pictus* |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 3: Average sewage indicator concentrations and densities per sampling location | | | | | | | | | |
| Site | NH4 mg/dm3 | NO3 mg/dm3 | PO4 mg/dm3 | Caffeine | Acetaminophen | Paraxanthine | Cotinine | Distance weighted population | Categorical distance weighted population |
| BK-1 | 0.003 | 0.085 | 0.054 | 0.011 | 0.001 | 0.002 | 0 | 2304.04 | High |
| BK-2 | 0.003 | 0.085 | 0.052 | 0.007 | 0.001 | 0 | 0 | 1891.56 | Mod |
| BK-3 | 0.068 | 0.09 | 0.045 | 0.003 | 0.001 | 0 | 0 | 1231.23 | Mod |
| BGO-1 | 0.0145 | 0.085 | 0.044 | 0 | 0.002 | 0 | 0 | 838.54 | Low |
| BGO-2 | 0.001 | 0.08 | 0.0385 | 0 | 0.001 | 0 | 0 | 611.91 | Low |
| BGO-3 | 0.001 | 0.09 | 0.044 | 0.005 | 0.003 | 0 | 0 | 624.45 | Low |
| OS-1 | 0.001 | 0.085 | 0.061 | 0 | 0.001 | 0 | 0.001 | 455.77 | Low |
| KD-1 | 0.0035 | 0.065 | 0.0375 | 0.003 | 0.001 | 0 | 0 | 662.42 | Low |
| KD-2 | 0.001 | 0.1 | 0.0445 | 0.001 | 0.001 | 0 | 0 | 720.55 | Low |
| MS-1 | 0.001 | 0.09 | 0.061 | 0.064 | 0.035 | 0.015 | 0 | 903.67 | Low |
| SM-1 | 0.001 | 0.085 | 0.1475 | 0.042 | 0.012 | 0.005 | 0 | 2146.22 | Mod |
| LI-1 | 0.004 | 0.08 | 0.0385 | 0.05 | 0.04 | 0.006 | 0.002 | 5403.21 | High |
| LI-2 | 0.091 | 0.095 | 0.0775 | 0.001 | 0.007 | 0 | 0 | 14792.51 | High |
| LI-3 | 0.0035 | 0.08 | 0.077 | 0.027 | 0.002 | 0.002 | 0.003 | 29511.73 | High |
| EM-1 | 0.1125 | 0.185 | 0.092 | 0.029 | 0.014 | 0.002 | 0 | 3389.95 | High |
| OS-2 | 0.001 | 0.08 | 0.078 | 0.033 | 0.001 | 0.004 | 0.003 | 4340.00 | High |
| OS-3 | 0.001 | 0.08 | 0.0795 | 0.001 | 0.001 | 0 | 0 | 1221.42 | Mod |

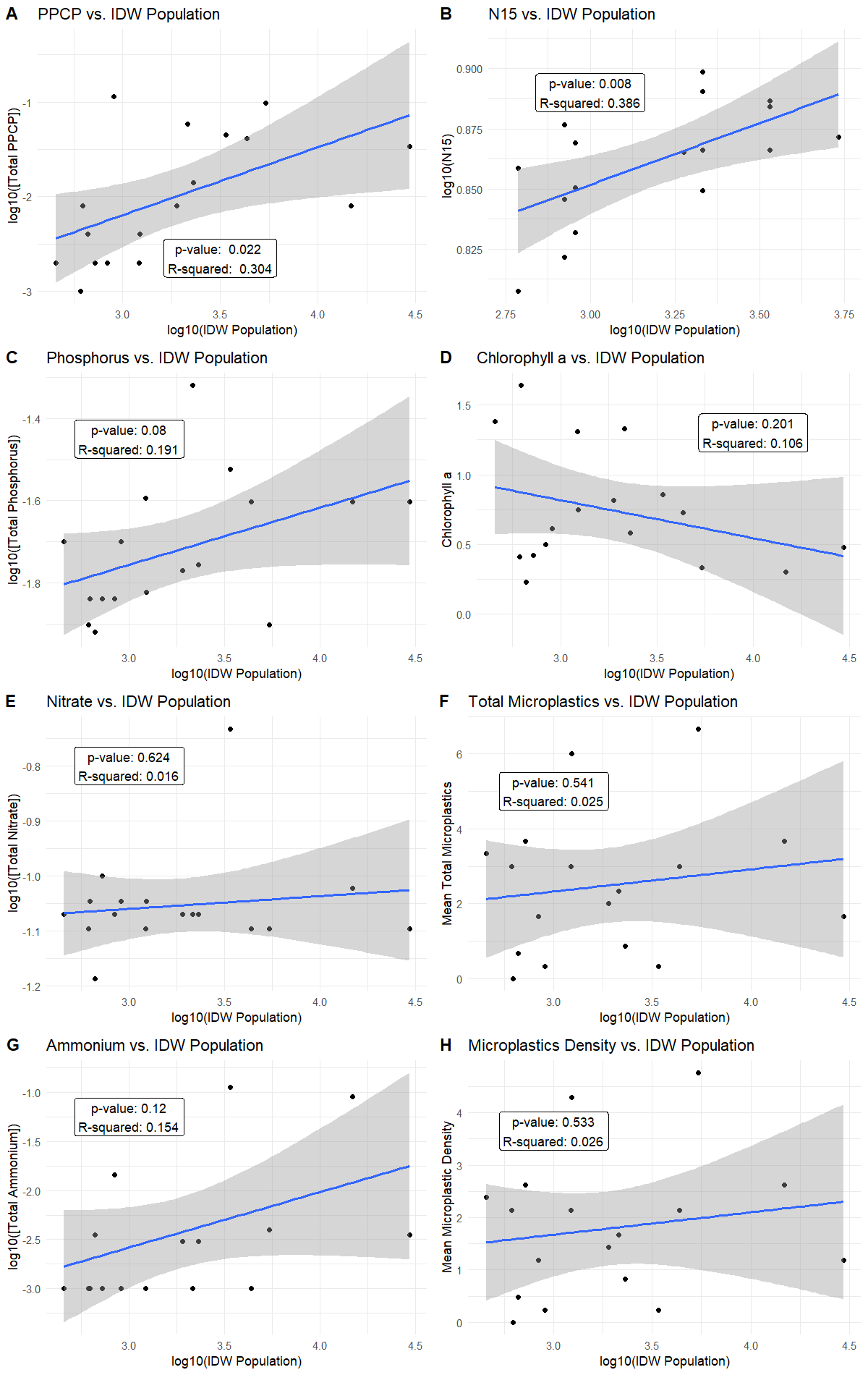


Figure 2: Linear models of total PPCP concentrations (A), N15 (B), phosphorus (C), Chlorophyll a (D), Nitrate (E), Total Microplastics (F), Ammonium (G), and Microplastic Density (H) regressed against log-transformed IDW population. Total PPCP concentrations (A), N15 (B), and phosphorus (C) produced significant models. Chlorophyll a (D), Nitrate (E), Total Microplastics (F), Ammonium (G), and Microplastic Density (H) did not produce significant models.

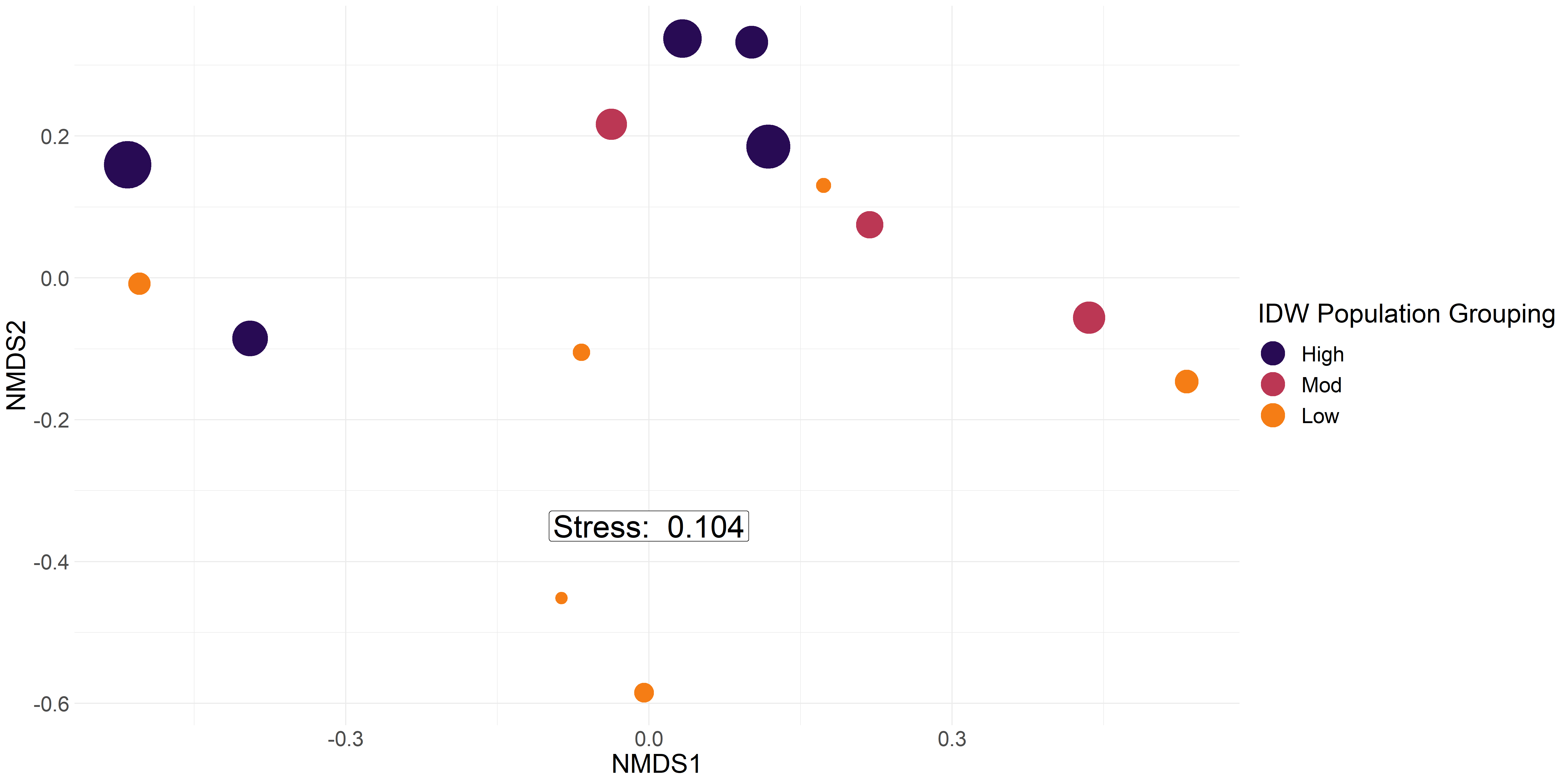


Figure 3: Periphyton abundance NMDS with bray-curtis dissimilarity. Labels are sized by log10 IDW population and colored by sites with high (purple), moderate (pink), and low (orange) IDW population values. PERMANOVA confirmed the three groups to be significantly different (p = 0.023). Sites with a higher IDW population value tended to be more associated with filamentous algal groupings, whereas moderate and low values were more associated with diatom abundance.

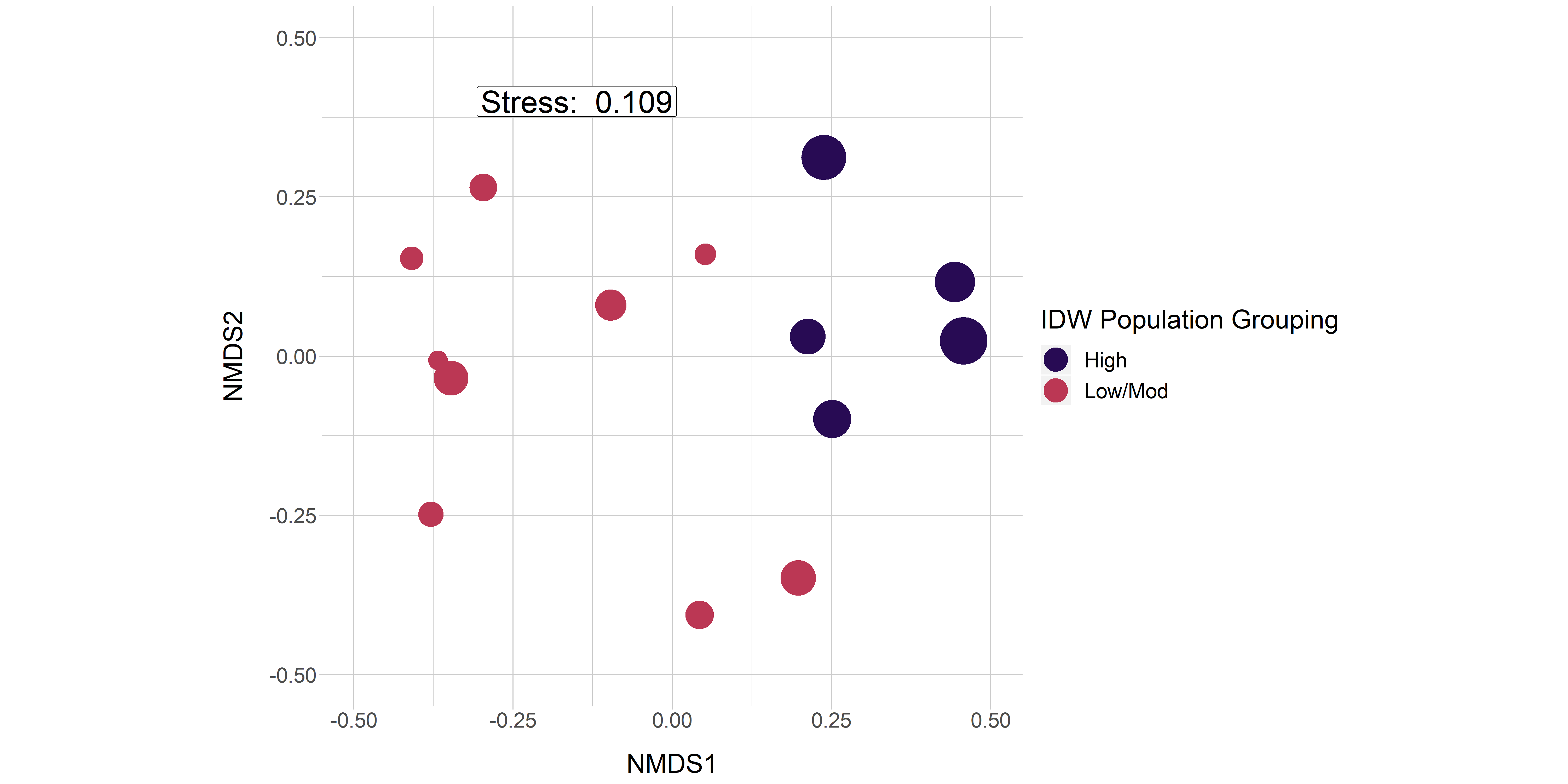


Figure 4: Macroinvertebrate abundance NMDS with bray-curtis dissimilarity. Sampling location labels are sized by log-transformed IDW population with major groups including low (orange), moderate (pink), and high (purple) IDW population. PERMANOVA confirmed the three groups to be significantly different (p = 0.002). Sites with a higher IDW population values tended to be associated with amphipod and leech taxa (see Table 2), whereas sites with lesser IDW population values were more associated with increased mollusc abundance (see Table 2).

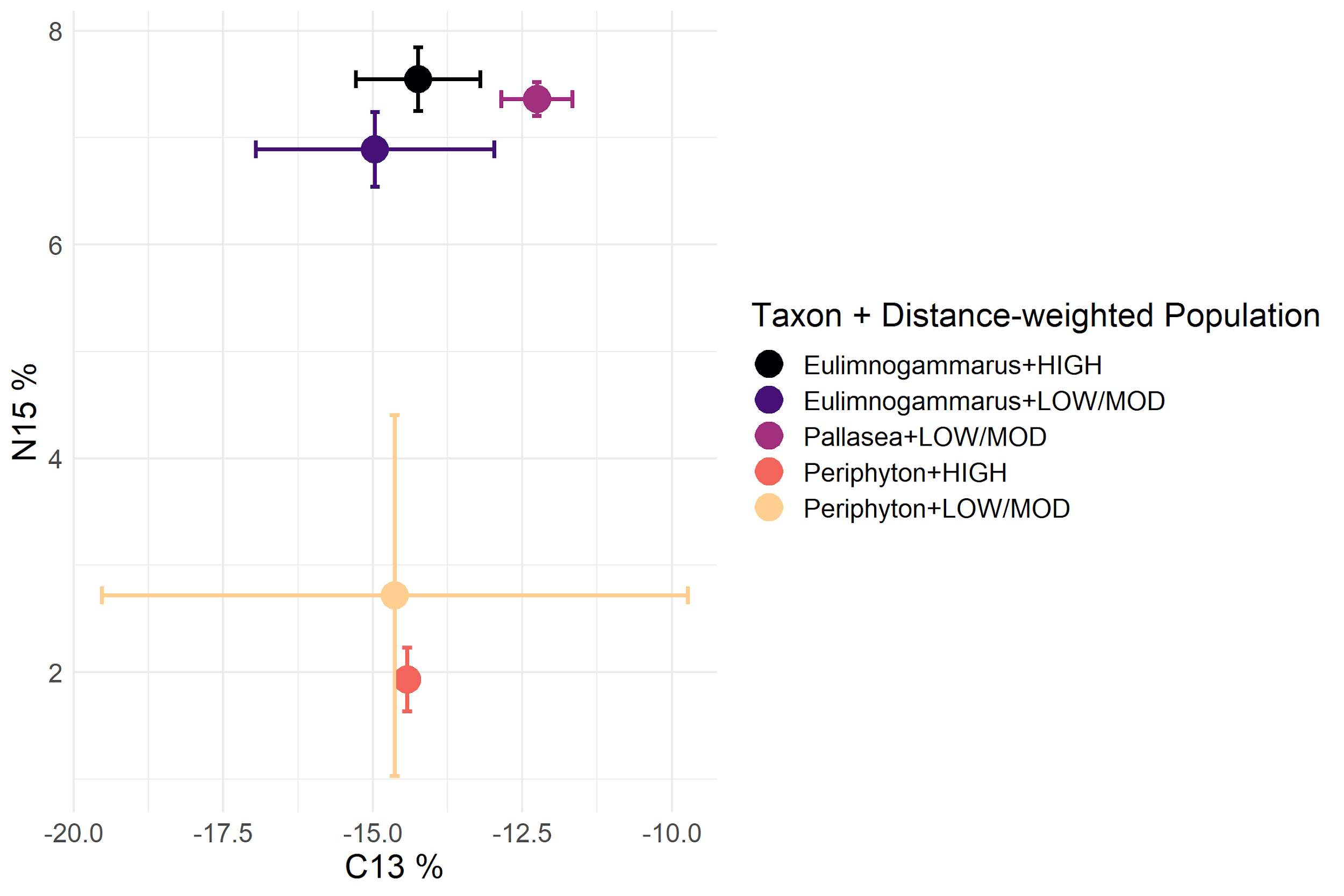


Figure 5: Biplot of mean and standard deviation C13 and N15 stable isotope values for littoral amphipods and periphyton, grouped by categorical IDW population (Table 3). In general, periphyton did not differ in C13 or N15 signatures with increasing IDW population, whereas *Eulimnogammarus* amphipods increased N15 signatures with increasing IDW population. *Pallasea* signatures differed from *Eulimnogammarus* most likely because Pallasea tends to remain in the nearshore area, whereas *Eulimnogammarus* will regularly migrate the deeper zones (Taakhteev & Didorenko, 2015).

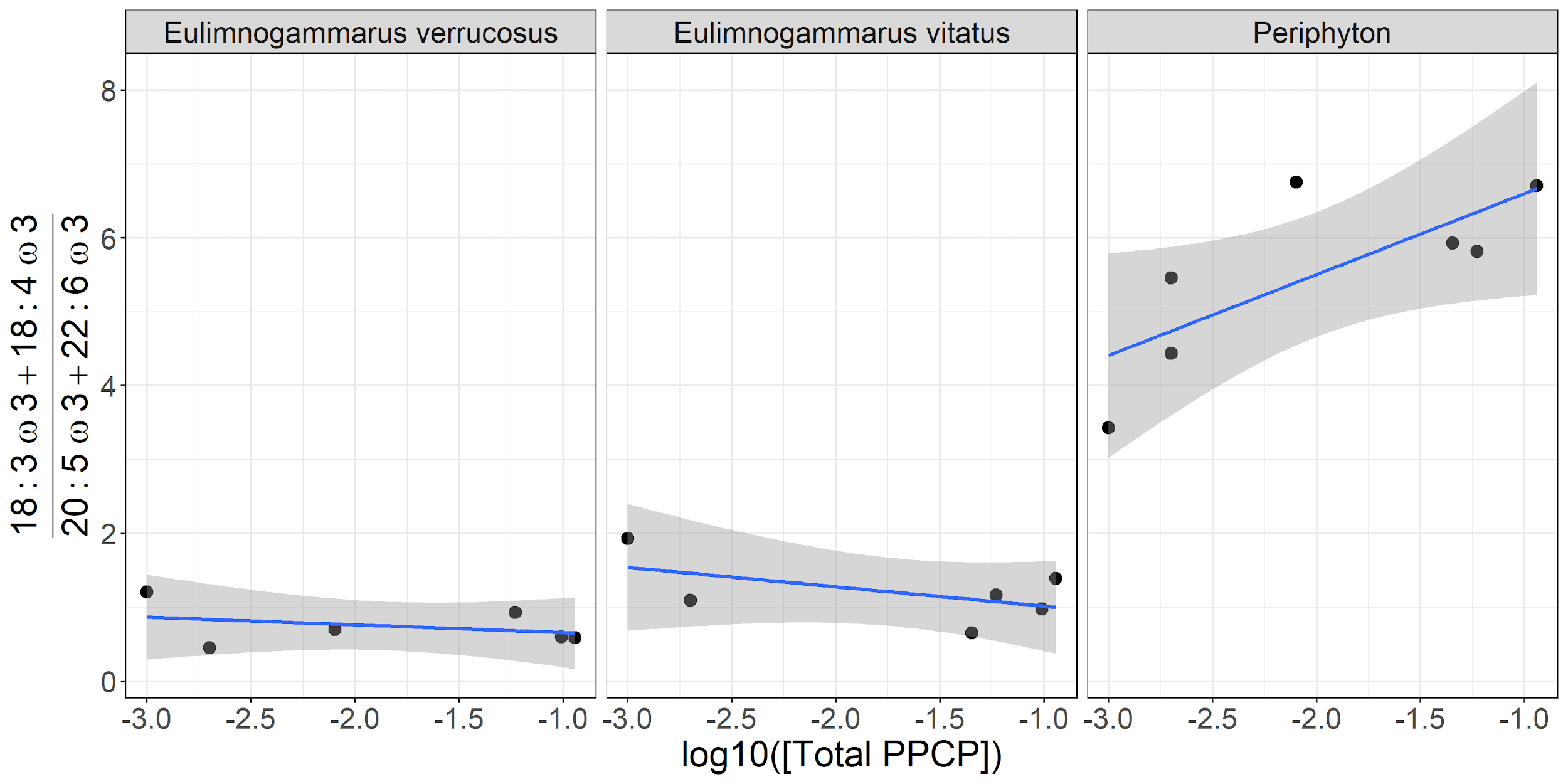


Figure 6: Ratio of 18:3ω3 and 18:4ω3 relative to 20:5ω3 and 20:6ω3 as a function of log -transformed total PPCP concentrations. The fatty acids 18:3ω3, 18:4ω3, 20:5ω3 and 20:6ω3 are all essential fatty acids (EFAs), which are prone to accumulate in organisms and mainly synthesized in primary producers. Because 18:3ω3 and 18:4ω3 are mainly found in filamentous algae whereas 20:5ω3 and 20:6ω3 tend to be associated with diatoms, our ratio also serves as a filamentous:diatom indicator. Periphyton ratios tend to increase with increasing total PPCP concentration, which corroborates our periphyton community abundance results (p = 0.05; Figure 3). Grazing amphipod ratios, however, remain relatively constant over a range of PPCP concentrations, implying two potential causes. First, amphipods could be selectively grazing to maintain a consistent ratio across sewage gradients. This potential cause would also imply that certain periphyton taxa, especially diatoms, would be experiencing increased grazing pressure at higher sewage concentrations. Second, amphipods could graze on filamentous algae and then expend their own energy to upgrade 18:3ω3 and 18:4ω3 to 20:5ω3 and 20:6ω3. In both of these scenarios, amphipods are able to maintain EFAs that are beneficial to living in cold environments despite necessary energetic trade-offs for remaining in areas with higher sewage concentrations.

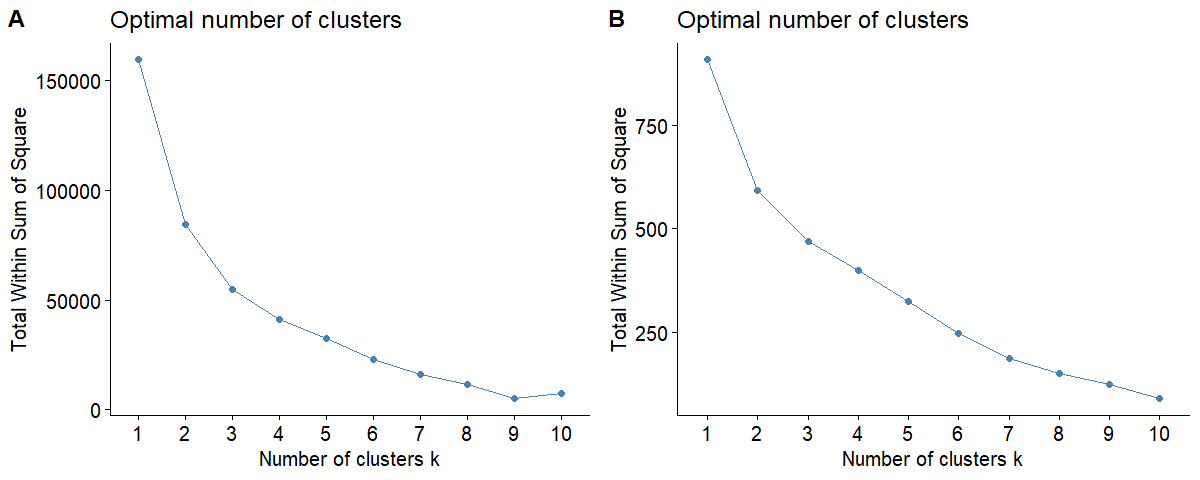


Figure S1: WSS for increasing number of clusters for periphyton (A) and invertebrate (B) community data. In the case of periphyton data, WSS decreases most markedly with three clusters, whereas invertebrate community abundance is best described by two clusters.

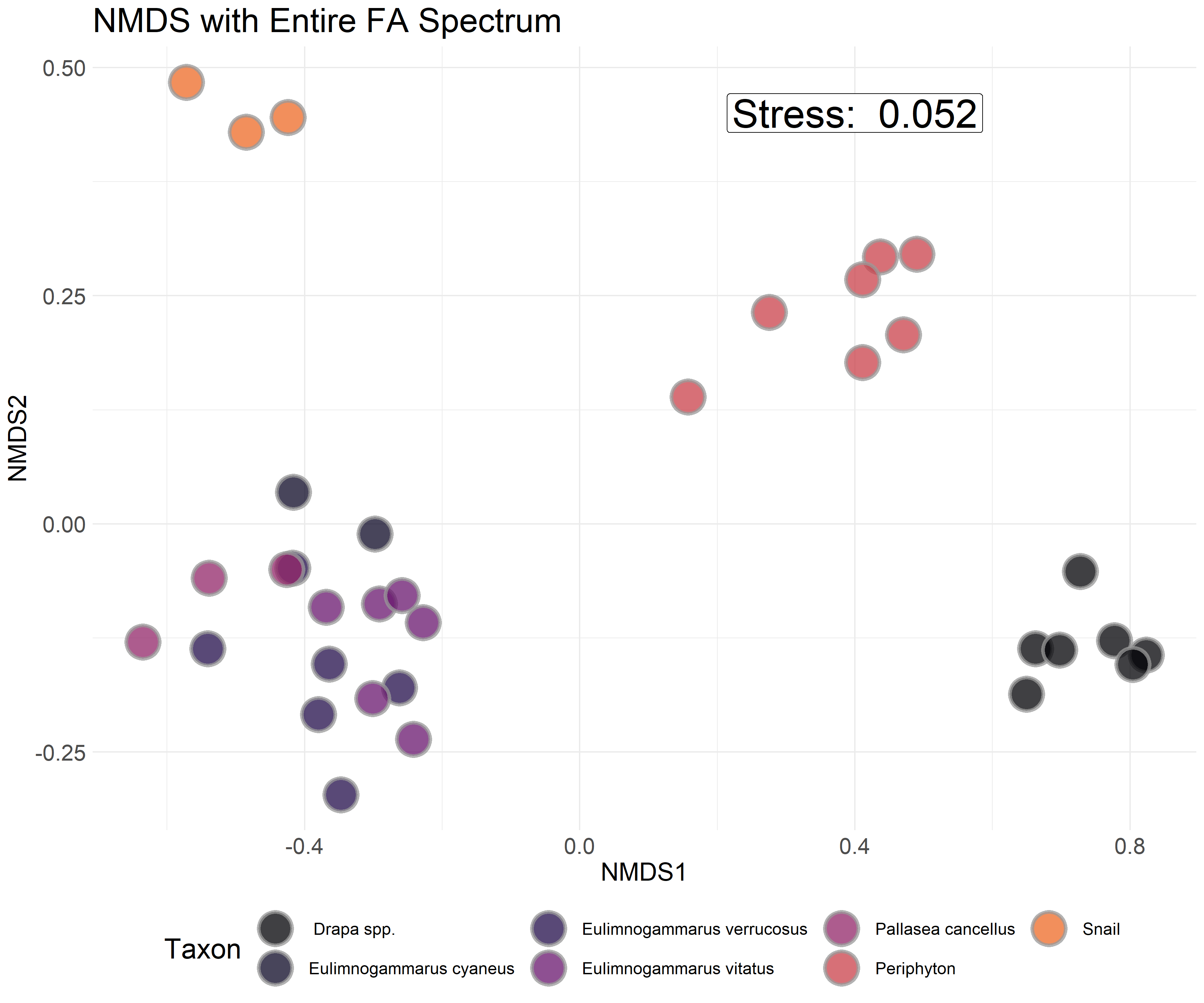


Figure S2: NMDS with bray-curtis dissimilarity of proportional fatty acid compositions for each macroinvertebrate and primary producer collected. *Eulimnogammarus* and *Pallasea* are endemic amphipod genera. *Drapa* spp. is an endemic filamentous algae. Snails were not identified to species prior to fatty acid analysis. Interspecific variation in fatty acid composition tended to be larger than intraspecific variation, implying that fatty acid signatures were largely species-specific and not environmentally driven.

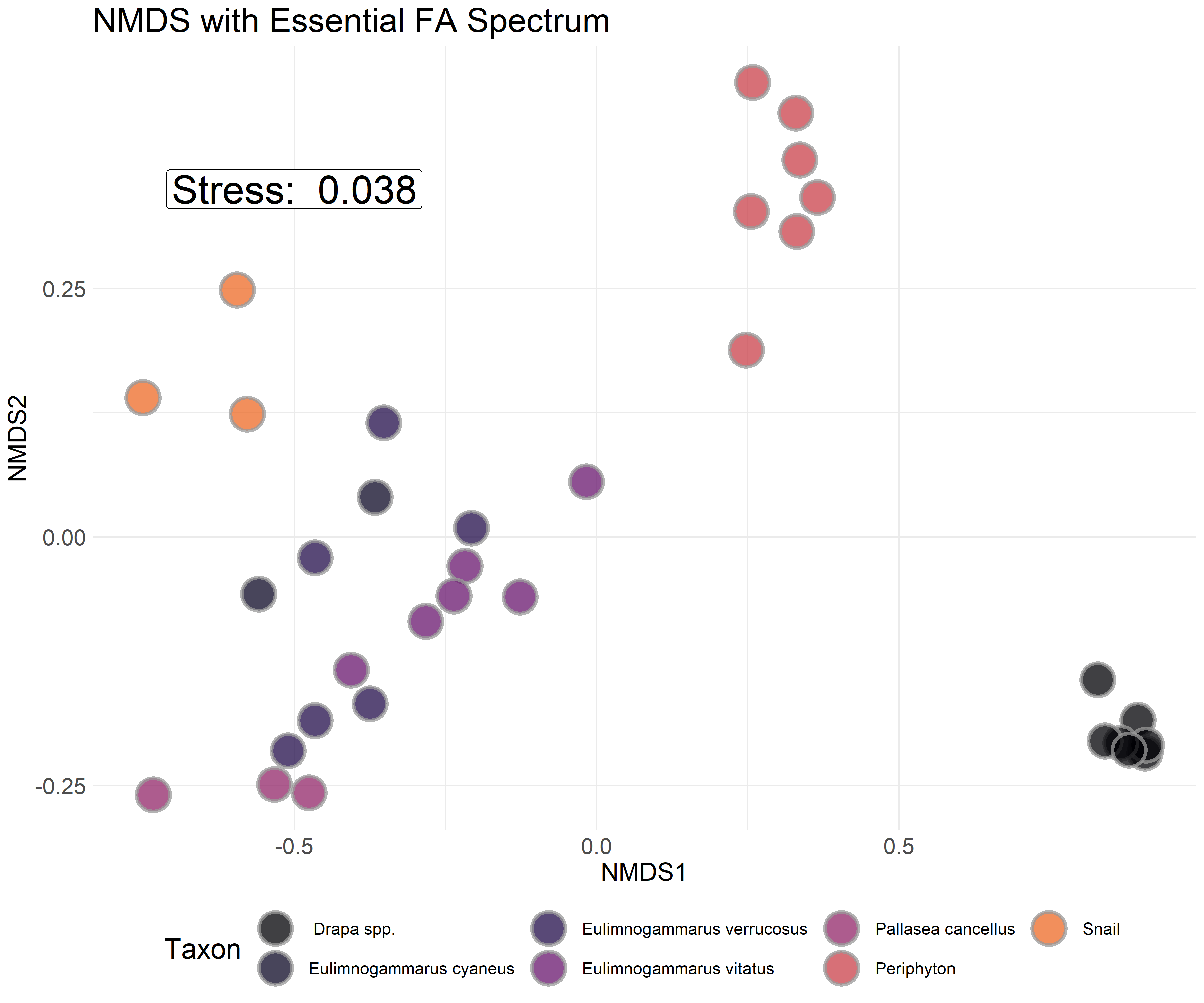


Figure S3: NMDS with bray-curtis dissimilarity of proportional essential fatty acid compositions for each macroinvertebrate and primary producer collected. *Eulimnogammarus* and *Pallasea* are endemic amphipod genera. *Drapa* spp. is an endemic filamentous algae. Snails were not identified to species prior to fatty acid analysis. Interspecific variation in fatty acid composition tended to be larger than intraspecific variation, implying that fatty acid signatures were largely species-specific and not environmentally driven.