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Multiyear Patterns in Benthic Algal Fatty-Acid Compounds Under Agricultural Stress

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Abstract: Benthic algae are the nutritional base of most freshwater food webs. Here we consider the nutritional contribution of benthic algae in the form of fatty acids (FAs) over multiple seasons and years. Our study was conducted in the Upper Delaware River watershed (New York, USA), where best-management practices (BMPs) had been implemented over varying numbers of years (BMP age) to mitigate effects of agricultural activities in the watershed. We defined 4 BMP categories by the presence and duration of management used in each stream drainage prior to initiating our study: 1) reference (unaffected), 2) no BMP (agriculturally affected), 3) new (agriculturally affected, BMP ≤ 2 y), and 4) old (agriculturally affected, BMP ≥ 3 y). Our results indicate that BMPs were able to reduce agricultural effects, as evidenced by a significantly lower benthic algal total FA content in reference than in agricultural streams. However, FA content did not differ between BMP-age categories, a result suggesting that BMPs were rapidly effective (within 1–2 y). Among essential molecules, amount of essential FA precursors (α-linoleic acid 18:3ω3 and linolenic acid 18:2ω6) were inversely related to their derivatives (arachidonic acid 20:4ω6, eicosapentaenoic acid 20:5ω3, docosahexaenoic acid 20:6ω3) across BMP-age categories. These outcomes suggest that BMP practices are important for maintaining the quality of the New York City metropolitan area water supply.
Key words: agriculture, benthic algae, bioassessment, BMPs, fatty acids, streams

Agricultural activities can profoundly alter the physical, chemical, and biological properties that constitute stable and healthy stream ecosystems. Their effects include elevated sediment loads, dissolved nutrients, and various environmental contaminants (Ehrman and Lamberti 1992, Strayer et al. 2003). The upper Delaware River system in New York State is a large (~2990 km^2) and important watershed affected by agriculture. Hundreds of streams draining this landscape feed a series of reservoirs that supply drinking water for the New York City region, a population of ~9 million people. Agricultural activities, which contribute nutrients to receiving streams, could lead to eutrophication of these reservoirs and, thereby, impair drinking-water quality (Boëchat et al. 2011). Regression models have shown that agricultural land use in the upper Delaware system is a major factor contributing to greater concentrations of total N and total P in streams, an effect that is proportional to the % agricultural land in a local drainage (Mehaffey et al. 2005).

Farming and forestry are important for the economic base of this region, so a conservation program was initiated in 1999 by the Watershed Agricultural Council (WAC) to create economically viable watershed protection based on best-management practices (BMPs) mainly in the form of setbacks, fencing, and riparian buffer zones (Pires 2004). BMPs are used in many agricultural watersheds to ameliorate negative effects and to restore stream ecosystems (Osborne and Kovacic 1993, Ice 2004, Grolleau and McCann 2012). The WAC has deployed >350 whole-farm plans in the broader watershed. BMPs implemented in headwater streams to mitigate adverse effects on water quality are focused specifically on elevated nutrients and other contaminants that originate from agricultural runoff (Bishop et al. 2005, Smith and Porter 2010).

Stream-associated BMPs are effective in minimizing many of the water-quality problems that affect public water supplies (Brannan et al. 2000, Bishop et al. 2005, Gitau et al. 2008) and are cost effective (Grolleau and McCann 2012). Better land management and improved water quality also lead to healthier biological communities and ecosystems (Allan 2004, Yates et al. 2007). Many assessments of BMPs have focused on the biodiversity of macroinvertebrates (Wat-

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zin and McIntosh 1999, Yates et al. 2007, Gabel et al. 2012), fish (Wang et al. 2006a), or both (Nerbonne and Vondracek 2001, Wang et al. 2006b, Yates and Bailey 2010). As a whole, these studies have shown improvements in fish and benthic macroinvertebrate metrics, based on greater biological diversity and abundance, improved bank stability, and overall stream habitat quality. Algae-based assessments of BMPs in streams are far less common. Of these, most have been studies of the effects of agricultural BMPs on reducing algal biomass, either in response to decreasing eutrophication of receiving waters (e.g., Brannan et al. 2000, Rasouli et al. 2014) or minimizing nuisance accumulations in the streams themselves (e.g., Dodds and Welch 2000, Carr et al. 2005, Jack et al. 2006). Investigators typically use before–after control–impact approaches to quantify BMP effectiveness. For example, significant reductions in the % cover of metaphyton in the littoral area of Conesus Lake (New York) was recorded after the introduction of BMPs to tributary streams and was attributed to lower concentrations of $NO_3^- + NO_2^-$ and soluble reactive P (SRP) in stream water (Bosch et al. 2009).

A key question emerging from these studies is whether the use of BMPs also affects the quality of basal food resources in streams, as reflected in the nutritional value of algal assemblages. Algal assemblages are of greater importance for higher trophic levels than was previously understood (Thorp and Delong 2002, McNeely et al. 2007, Brett et al. 2017). Algae have low C : N and C : P ratios (Frost et al. 2002) and are the primary source of fatty acids (FAs) for aquatic consumers (Brett and Müller-Navarra 1997, Torres-Ruiz et al. 2007, 2010). Most consumers have the ability to synthesize short-chain FA compounds, but many lack the ability to extend beyond 18C or to desaturate longer-chain compounds to create metabolically important polyunsaturated FAs (PUFAs) (Stanley-Samuelson 1994, Olsen 1999, Torres-Ruiz et al. 2010). Compounds particularly important to consumers are essential ω3 PUFAs such as α-linoleic acid (ALA 18:3ω3), eicosapentaenoic acid (EPA 20:5ω3), and docosahexaenoic acid (DHA 22:6ω3), and essential ω6 PUFAs, such as linolenic acid (LIN 18:2ω6) and arachidonic acid (ARA 20:4ω6) (Napolitano 1999, Parrish 2009). In fish larvae, these compounds are essential for organismal growth, metabolic regulation, neural tissue development, and later reproductive function (Olsen 1999). Benthic algae also provide more total lipids and protein on a per mass basis than terrestrial-based detritus (Lamberti 1996, Torres-Ruiz et al. 2007). For these reasons, FA compounds are increasingly being used as biomarkers of algal nutritional quality, and their amounts have been used to assess effects of human disturbance (Larson et al. 2013, Boëchat et al. 2014).

The nutritional composition of algal assemblages also varies temporally. Benthic algae in a forested stream in New York State (USA) had greater total amounts of FAs during the spring and least in the summer, but seasonal trends varied depending on the individual FA compound (Torres-Ruiz et al. 2007). The FA composition of periphyton differed in important ways in minimally disturbed headwater streams in Pennsylvania (USA) over the course of a year, with greatest amounts of Σω3 and Σω6 FAs during summer and autumn (Honeyfield and Maloney 2014). Such spatial and temporal variations in the nutritional quality of basal food sources suggest long-term implications for managed aquatic ecosystems in agricultural landscapes.

Our goal was to characterize longer-term patterns in FA composition of benthic algal assemblages in streams affected by agricultural activities with varying degrees of BMPs. We addressed this question in an agricultural region of the upper Delaware River watershed by comparing 4 sets of headwater streams: agricultural streams with no protection, newly installed BMPs, or established BMPs, and reference sites. The changes in taxonomic composition of benthic algal assemblages in relation to FA content have been analyzed separately (Whorley and Wehr 2016). Here, we predicted that: 1) the increasing age of a BMP would result in increased benthic algal FA content and 2) seasonal changes in stream conditions would be reflected in the seasonal patterns in total FA and specific essential FA compounds in the benthic algae.

METHODS

Stream selection

Study streams were 1st- to 3rd-order in size (≥1 m wide, ≥5 cm deep) with current velocity >5 cm/s. Each sampling site consisted of a riffle stretch with predominantly cobble substrata, where boulders (>1 m major dimension) made up <20% of the stream bed. Selection of sites also required obtaining permission from farm landowners for access and approval to sample each stream (Fig. 1). The aim of the study design was to quantify the effectiveness of BMP implementation based on presence and duration of implementation. Temporal categories were chosen to accommodate the array of sites currently in existence in this region and were based, in part, on an earlier bioassessment (involving fewer sites) in which the investigators used diatom indicators and suggested differences in BMP effectiveness (Gabel et al. 2012).

BMPs in our study included riparian reconstruction, stream-side fencing, and barnyard improvements (cement floors and roofing). Agricultural stressors were row-crop production (corn and hay), dairy cattle and goats, and equestrian boarding. We sampled monthly from April through November 2011 to 2014. Stream sites were defined (in 2011 at the start of this study) based on levels of physical and temporal difference and were assigned to 1 of 4 agricultural disturbance/mitigation categories, according to information provided the Watershed Agricultural Council (WAC 2011): 1) reference (Ref): unaffected streams draining nonagricultural, nonresidential land ($n = 5$), 2) no BMP (Non): agriculturally affected streams lacking BMPs ($n = 4$), 3) new: ag-

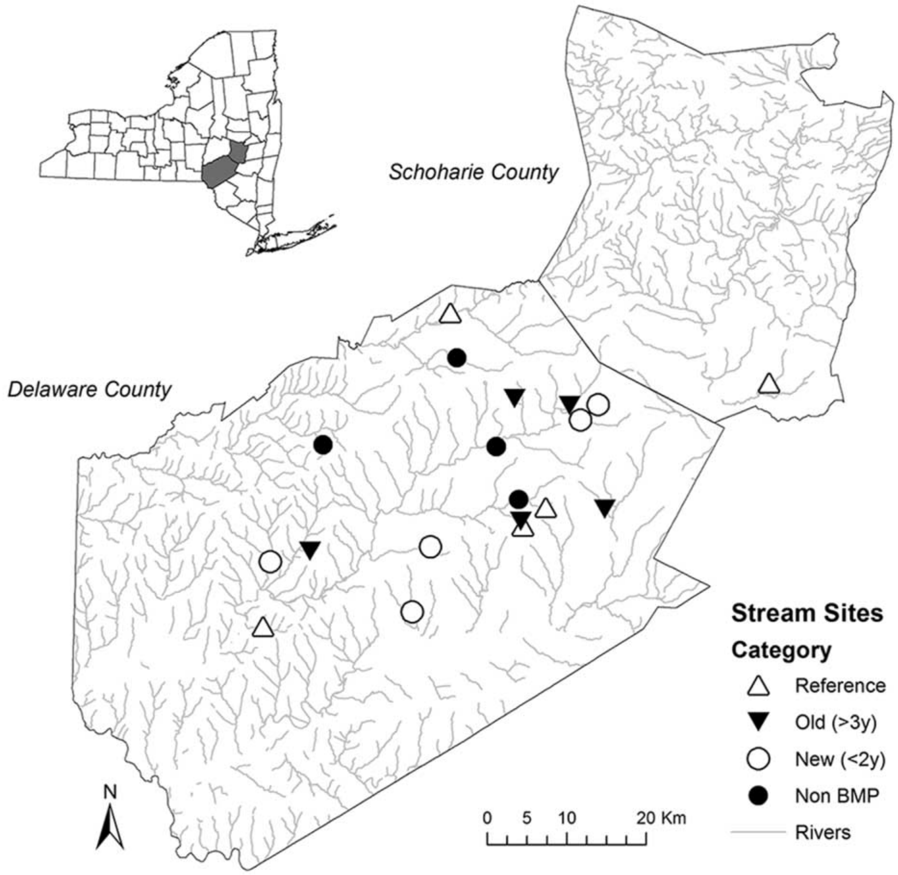

Figure 1. Map of study stream locations and categories in the Upper Delaware River watershed, New York State. Best management practice (BMP) age is based on age in 2011.

riculturally affected streams with ≥1 BMP in place for ≤2 y (n = 5), and 4) old: agriculturally affected streams with ≥1 BMP in place for ≥3 y (n = 5).

Water chemistry and stream characteristics

We collected stream water, filtered it in the field through a 0.2-μm pore-size syringe filter, and preserved it with H_2SO_4 (pH < 2.0) in acid-washed polypropylene tubes (USEPA 1987). We analyzed water for soluble reactive P (SRP), NO_3^-/NO_2^-, and NH_4^+ on an Astoria–Pacific (Clackamas, Oregon) A2 nutrient analyzer using a H2O Check™ kit from Astoria–Pacific (USEPA 1987). We collected, filtered, and preserved it with HCl (pH < 2.0) for dissolved organic C (DOC) concentration. We analyzed these samples on a Shimadzu TOC-L analyzer (Shimadzu, Columbia, Maryland) as nonpurgeable organic C (EPA method 415.3; Potter and Wimsatt 2005). We collected an unfiltered water sample for total suspended solids (TSS) in a 1-L polypropylene bottle and later filtered it through a GF/F filter (GE/Whatman, Buckinghamshire, UK). We calculated TSS mass after drying at 80°C for ≥48 h. We evaluated canopy cover with a concave densiometer while facing upstream, downstream, and each bank.

Benthic algae collection and analysis

We collected benthic algae from each site by arbitrarily selecting 3 cobbles 6 to 12 cm in diameter. Following EPA protocols, we scrubbed cobbles with a toothbrush on all above-streambed surfaces to remove attached benthic algae (Stevenson and Bahls 1999). We then measured pooled benthic algal volume and stored samples in acid-washed (10% HCl) polypropylene containers on ice while in the field and in a 4°C refrigerator with caps vented in the laboratory. We processed benthic algal samples <48 h after collection. We extracted Chl *a* samples in $MgCO_3$-neutralized 90% acetone and processed them on a Shimadzu UV-1800 spectrophotometer (Columbia, Maryland). We corrected for phaeo-pigments using 0.1 M HCl (Lorenzen 1967, Jeffrey and Humphrey 1975). We analyzed algal N concentrations on a Thermo Scientific Flash 2000 Organic Elemental Analyzer (Pittsburg, Pennsylvania). We processed algal P concentrations by $MgCO_3$ digest, followed by combustion at

450°C and resuspension in 0.2 M HCl. Samples were then diluted and processed as SRP (Solorzano and Sharp 1980).

We filtered 30 to 50 mL of pooled benthic algal material for FA extraction onto precombusted GF/F filters (GE/Whatman, Buckinghamshire, UK) and stored the filters at −20°C in chloroform-washed borosilicate test tubes after flushing samples with N_2 gas. We extracted samples with chloroform : methanol (2 : 1), methylated using BF_3, and transferred to a hexane solvent (Parrish 1999, Torres-Ruiz et al. 2007, Whorley and Wehr 2016). We used an internal standard of nonadecanoic acid (19 : 0) and blank hexane samples to test methylation efficiency and to assess consistency among sample runs. We analyzed and quantified samples on a Shimadzu (Tokyo, Japan) GC-2014 fitted with a capillary column (Omegawax-320, 30 m × 0.32 mm × 0.25 μm film thickness; Supelco®, Bellefonte, Pennsylvania). The temperature program has an initial injection into a splitless inlet at a temperature of 100°C, followed by 1 h ramping to 260°C in increments of 10°C/10 min with He as the carrier gas to a flame ionization detector. We made the standard dilution series for analysis and standard curves of the FA compounds Supelco 37 component FA methyl ester (FAME) mix. Quantification was limited to compounds ≥18C because many biologically important FAs are derived from 18C base molecules (Stanley-Samuelson 1994). Detection limits of the GC were set to identify peaks that corresponded to a minimum content of 0.011 to 0.024 mg/m^2, depending on the size of the molecule. Common FA terms can be found in Table 1, and a full list of enumerated compounds is available in Table S1.

Data analyses

We checked all data for normality assumptions of subsequent tests (Shapiro–Wilk analysis) and log(x)-transformed them if necessary. However, we present untransformed data.

Table 1. Quick guide to commonly used abbreviations for the analysis and discussion of benthic algal fatty-acid structural categories and key compounds. All fatty-acid compounds were identified from a Supelco FAME 37 standard.

Abbreviations	Definition
BMP	Best management practice
FA	Fatty acid
PUFA	Polyunsaturated FA
Σω3	Total ω-3 FAs
Σω6	Total ω-6 FAs
Σother	Total FAs, excluding ω3 and ω6
ALA	α-linolenic acid (18:3ω3)
EPA	Eicosapentaenoic acid (20:5ω3)
DHA	Docosahexaenoic acid (22:6ω3)
LIN	Linoleic acid (18:2ω6)
ARA	Arachidonic acid (20:4ω6)

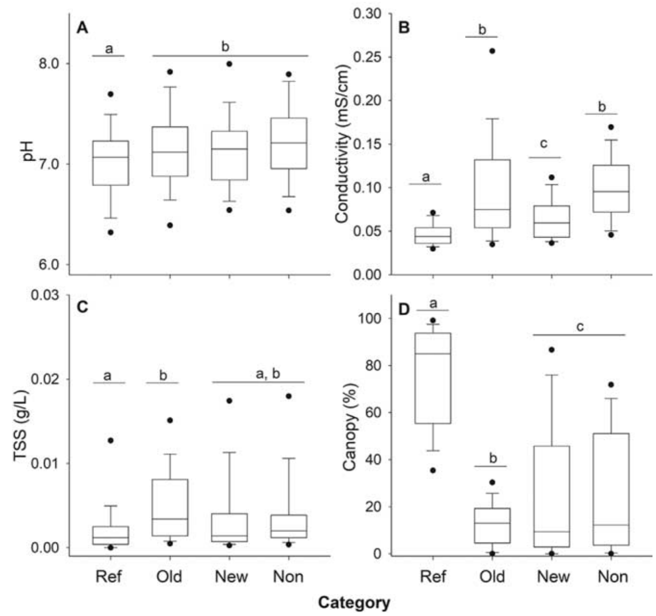

Figure 2. Box-and-whisker plots of pH (A), conductivity (B), total suspended solids (TSS) (C), and % canopy cover (D) in streams by category (Ref = reference, old = agricultural with best management practice (BMP) installed ≥3 y, new = agricultural with BMP installed ≤2 y, non = agricultural without BMP). Lines in boxes are medians, box ends are 1st and 3rd quartiles, whiskers are 10th and 90th percentiles, and circles are outliers. Box plots sharing a letter are not significantly different (Tukey's Honest Significant Difference, $p > 0.10$).

We aggregated FA compounds into groups of total, Σω3, Σω6, and Σothers (neither ω3 nor ω6). We used analyses of variance (ANOVAs) to test for differences in stream characteristics and FA amounts among stream categories, months, and years (Sokal and Rohlf 1995). We followed ANOVAs that yielded significant differences among months of years with 1-way ANOVAs to test for differences in FA content among stream categories. We used Tukey's Honest Significant Difference (HSD) tests to identify which stream categories and sampling dates differed. We assessed the relationships among algal biomass, stoichiometry, and FA content with a Spearman's rank correlation. We used principal components analysis (PCA) and discriminant analysis (DA) to summarize monthly averages of stream characteristics, summed groups of FA compounds, and individual essential FA compounds (Table 1). We used these loadings to plot individual sites based nonmetric multidimensional scaling (NMDS). We set α = 0.10 because of the inherently high variability in this field study. We conducted all statistical analyses in SYSTAT 13 (Systat Software, Chicago, Illinois).

RESULTS

Stream conditions

Stream categories differed with regard to key water-chemistry variables. pH in reference streams averaged 7.03,

Table 2. Monthly mean (±SE) stream-water nutrient concentrations (μg/L) over the duration of the study separated by stream category. July–November contains all measurements from 2011–2013. April–June contains all measurements from 2012–2014. Analysis of variance results are in the bottom of each stream category and variable. Bold = significant difference among months (Tukey's Honest Significant Difference, $p < 0.1$). Ref = reference streams, old = agricultural streams with best management practice [BMP] installed >3 y, new = agricultural streams with BMP installed ≤2 y, non = agricultural streams without BMP, n/a = not applicable.

Nutrient	Month	Ref	Old	New	Non
NO_3^--N μg/L	April	274.6 (45.3)	436.4 (49.1)	355.4 (41.4)	392.7 (43.0)
	May	210.7 (31.1)	284.9 (31.7)	185.9 (35.3)	216.0 (17.3)
	June	183.5 (20.9)	307.3 (45.7)	164.4 (28.7)	183.4 (21.7)
	July	218.5 (28.6)	466.7 (81.6)	205.4 (34.5)	291.9 (33.3)
	August	225.4 (16.4)	515.6 (104.6)	171.8 (30.7)	189.6 (33.2)
	September	228.9 (21.4)	437.1 (153.1)	209.0 (60.1)	128.6 (24.2)
	October	125.3 (25.7)	290.6 (79.5)	124.8 (33.6)	115.1 (24.2)
	November	237.3 (37.8)	477.6 (117.8)	244.7 (41.3)	1045.8 (775.5)
	$F_{7,107}$ (p)	1.637 (0.200)	2.078 (0.111)	**5.032 (0.004)**	1.007 (0.464)
	Differences	n/a	n/a	**Apr > All**	n/a
NH_4^+-N (μg/L)	April	28.5 (1.6)	23.4 (3.3)	32.4 (3.7)	33.0 (2.6)
	May	35.5 (4.2)	34.7 (5.9)	35.7 (5.0)	41.8 (4.9)
	June	42.1 (8.2)	51.4 (6.6)	47.8 (3.6)	42.7 (3.1)
	July	32.9 (4.2)	37.3 (5.9)	47.0 (6.3)	48.5 (5.5)
	August	60.4 (5.7)	77.0 (11.7)	72.6 (9.7)	78.1 (10.1)
	September	16.2 (2.1)	24.0 (3.1)	23.5 (3.3)	27.0 (3.6)
	October	47.8 (6.9)	67.7 (11.7)	51.6 (7.9)	62.5 (7.9)
	November	29.3 (5.3)	30.5 (6.8)	38.9 (12.0)	34.1 (5.5)
	$F_{7,107}$ (p)	1.586 (0.214)	**2.451 (0.069)**	1.136 (0.392)	**3.111 (0.031)**
	Differences	n/a	n/a	n/a	**Apr, Sep, Nov < Aug**
SRP (μg/L)	Apr	18.5 (4.3)	10.1 (1.4)	9.2 (1.6)	8.7 (0.8)
	May	7.4 (0.6)	8.7 (1.0)	9.9 (1.7)	9.5 (1.4)
	Jun	9.3 (0.6)	12.5 (1.2)	12.5 (1.6)	13.8 (1.9)
	Jul	13.0 (0.7)	18.1 (2.1)	17.6 (2.6)	50.6 (27.0)
	Aug	38.8 (21.9)	20.0 (1.9)	20.1 (2.3)	25.5 (2.5)
	Sep	12.2 (1.1)	15.7 (2.2)	12.2 (1.9)	20.3 (3.1)
	Oct	13.0 (1.1)	26.9 (15.2)	15.2 (2.4)	17.1 (2.1)
	Nov	6.5 (0.7)	6.8 (1.0)	7.6 (1.3)	8.7 (1.2)
	$F_{7,107}$ (P)	1.400 (0.275)	1.247 (0.338)	**5.467 (0.003)**	**2.241 (0.090)**
	Differences	n/a	n/a	**Apr, May, Nov < Aug**	**Apr, Nov < Jul**
				May, Nov < Jul	
DOC (μg/L)	April	5.1 (0.6)	7.7 (1.0)	8.6 (1.8)	8.3 (1.4)
	May	11.1 (1.5)	11.8 (2.0)	9.5 (0.7)	9.4 (1.5)
	June	8.5 (1.2)	12.4 (2.0)	11.7 (2.1)	10.9 (1.2)
	July	7.0 (0.8)	11.8 (1.3)	11.4 (1.0)	13.0 (1.6)
	August	8.1 (0.8)	12.3 (1.2)	9.5 (0.8)	12.5 (1.2)
	September	12.4 (3.7)	15.8 (2.8)	12.5 (1.9)	13.1 (1.1)
	October	9.1 (0.5)	13.0 (1.1)	12.7 (1.0)	15.7 (1.0)
	November	10.0 (0.9)	10.8 (1.1)	9.3 (0.7)	11.4 (0.7)
	$F_{7,107}$ (p)	1.093 (0.415)	1.605 (0.209)	0.958 (0.494)	1.658 (0.194)
	Differences	n/a	n/a	n/a	n/a

Table 3. Summary of discriminant analysis on stream characterization by both physicochemical measurements and fatty acid (FA) profile. Physicochemical conditions include all measured chemical and physical parameters of streams. FA profile includes all identified compounds ≥18C (see Table S1 for all compounds). See Table 2 for stream abbreviations. ID = stream category.

		Physicochemical conditions			Algal FA profile		
Stream category	Total number	Correct ID (%)	Variable	Canonical function (within s^2)	Correct ID (%)	Variable	Canonical function (within s^2)
Ref	115	81	Canopy	0.923	76	22:6ω3	0.703
Old	115	50	Conductivity	0.201	34	18:1ω9	0.475
New	112	51	DOC	0.109	34	20:4ω6	0.431
Non	92	20			32		

whereas pH in agricultural streams (with and without BMPs) averaged between 7.13 and 7.22 ($F_{3,430} = 3.767$, $p = 0.011$; Fig. 2A). Greater differences were observed in stream-specific conductivity ($F_{3,430} = 36.098$, $p < 0.001$) and TSS ($F_{3,430} = 3.941$, $p = 0.009$) (Fig. 2B, C). The specific conductivity of reference streams averaged 0.05 mS/cm, which was significantly lower than agricultural streams as a whole (Tukey's HSD, $p < 0.10$). Conductivity was significantly lower in new (0.06 mS/cm) than in old (0.10 mS/cm) and no BMP streams (0.10 mS/cm) (Tukey's HSD, $p < 0.001$). TSS of old BMP streams (mean = 6.5 mg/L) averaged >2× that measured in reference streams (2.5 mg/L) (Tukey's HSD, $p = 0.004$). No significant differences were observed between reference and any of the agricultural stream categories with

Table 4. Analysis of variance and Tukey's Honest Significant Difference (HSD; $p < 0.01$) comparing mean (±SE) seasonal taxonomic proportions among stream categories. Means are derived from the cumulative proportion of each taxonomic group within each stream category for an individual month, regardless of year. The proportions of taxa belonging to rhodophyte or chrysophyte divisions were summed (Rhod/Chrys). Significant results are in bold. See Table 2 for category abbreviations.

Stream category	Spring (April, May)	Summer (June, July, August)	Autumn (September, October, November)
Ref	Diatom: 0.71 (0.04)	Diatom: 0.72 (0.02)	Diatom: 0.76 (0.02)
	Chlorophyte: 0.16 (0.04)	Chlorophyte: 0.15 (0.02)	Chlorophyte: 0.12 (0.03)
	Cyanobacteria: 0.13 (0.02)	Cyanobacteria: 0.12 (0.02)	Cyanobacteria: 0.10 (0.02)
	Rhod/Chrys: 0.01 (0.01)	Rhod/Chrys: 0.01 (0.01)	Rhod/Chrys: 0.02 (0.01)
Old	Diatom: 0.80 (0.04)	Diatom: 0.76 (0.01)	Diatom: 0.65 (0.04)
	Chlorophyte: 0.09 (0.02)	Chlorophyte: 0.12 (0.010)	Chlorophyte: 0.13 (0.02)
	Cyanobacteria: 0.07 (0.01)	Cyanobacteria: 0.11 (0.01)	Cyanobacteria: 0.23 (0.03)
	Rhod/Chrys: 0.04 (0.03)	Rhod/Chrys: 0.01 (0.01)	Rhod/Chrys: 0.002 (–)
New	Diatom: 0.81 (0.02)	Diatom: 0.77 (0.03)	Diatom: 0.82 (0.03)
	Chlorophyte: 0.11 (0.02)	Chlorophyte: 0.09 (0.02)	Chlorophyte: 0.07 (0.02)
	Cyanobacteria: 0.08 (0.01)	Cyanobacteria: 0.14 (0.02)	Cyanobacteria: 0.11 (0.01)
	Rhod/Chrys: 0.001 (0.001)	Rhod/Chrys: 0.004 (0.003)	Rhod/Chrys: 0.003 (0.002)
Non	Diatom: 0.78 (0.02)	Diatom: 0.78 (0.02)	Diatom: 0.81 (0.01)
	Chlorophyte: 0.14 (0.01)	Chlorophyte: 0.13 (0.01)	Chlorophyte: 0.07 (0.01)
	Cyanobacteria: 0.08 (0.01)	Cyanobacteria: 0.09 (0.01)	Cyanobacteria: 0.12 (0.01)
	Rhod/Chrys: 0.003 (0.002)	Rhod/Chrys: 0.01 (0.002)	Rhod/Chrys: – (–)
ANOVA (F, p)	Diatom: $F_{3,20} = 2.163$, $p = 0.124$	Diatom: $F_{3,32} = 1.466$, $p = 0.242$	**Diatom: $F_{3,28} = 10.338$, $p < 0.001$**
	Chlorophyte: $F = 1.429$, $p = 0.264$	**Chlorophyte: $F = 2.937$, $p = 0.048$**	Chlorophyte: $F = 1.846$, $p = 0.162$
	Cyanobacteria: $F = 2.694$, $p = 0.074$	Cyanobacteria: $F = 2.168$, $p = 0.111$	**Cyanobacteria: $F = 8.743$, $p < 0.001$**
	Other: $F = 1.069$, $p = 0.385$	Other: $F = 0.266$, $p = 0.850$	Other: $t = 2.447$, $p = 0.328$
Tukey's HSD	Cyanobacteria: Ref > Old	Chlorophyte: Ref > New	Diatom: Old < All Others
			Cyanobacteria: Old > All Others

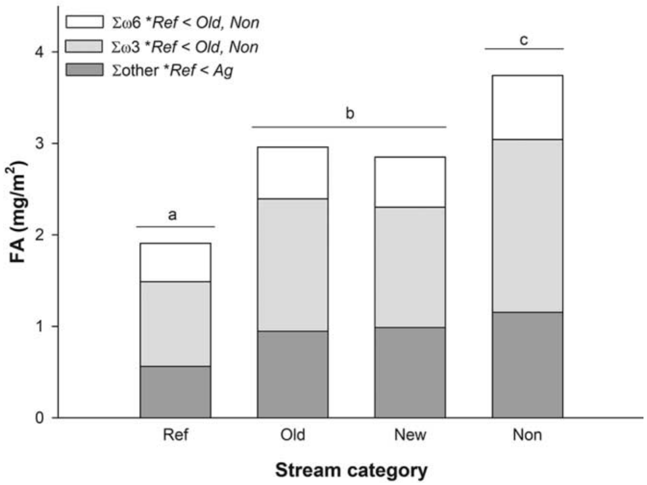

Figure 3. Fatty-acid (FA) category content (mg/m^2) by stream category averaged over the course of the entire study. Bars sharing a letter are not significantly different (Tukey's Honest Significant Difference [HSD], $p > 0.10$). * indicates significant differences among categories (Tukey's HSD, $p < 0.10$). Ag = agricultural. See Fig. 2 for stream categories.

respect to width, depth, temperature, current velocity, or dissolved O_2 ($p > 0.10$). Canopy cover in reference streams averaged 75.8%, whereas in agricultural streams as a whole, it averaged only 22.5% ($F_{3,430} = 160.29$, $p < 0.001$). Canopy cover was lowest in agricultural streams with old BMPs but did not differ between streams with new or no BMPs (Fig. 2D).

Nutrient concentrations differed significantly between reference and agricultural streams as a whole (Table 2). Average concentrations of NH_4^+ ($F_{3,430} = 2.116$, $p = 0.097$) and NO_3^- ($F_{3,430} = 4.124$, $p = 0.007$) differed among stream categories. Average NH_4^+ concentrations in reference streams (37.4 μg N/L) were ~20% lower than in agricultural streams with no BMPs (mean = 46.8 μg/L) (Tukey's HSD, $p = 0.093$). NH_4^+ concentration did not differ between streams with old and new BMPs. Streams with old BMPs had greater NO_3^- (400 μg N/L) than reference streams (Tukey's HSD, $p < 0.019$) and streams with new BMPs (Tukey's HSD, $p < 0.020$), but NO_3^- did not differ between streams with old and no BMPs. Average DOC in reference streams (~8.8 mg/L) was lower than and differed significantly from DOC in agricultural streams (mean = 11.34 μg/L) ($F_{3,430} = 7.878$, $p < 0.001$), but did not differ substantially among agricultural stream categories. SRP did not differ among stream categories ($F_{3,430} = 0.997$, $p = 0.394$). Despite occasional significant differences in nutrient concentrations between reference and agricultural streams, average concentrations of priority nutrients and other conditions were well below water-quality limits set for surface waters by various environmental monitoring agencies and other works establishing water-quality benchmarks (NYSDEC 1999, USEPA 2001, Dodds and Oakes 2004).

Stream nutrient conditions differed seasonally. SRP showed an increase in late summer and a smaller increase in late autumn in all categories. This seasonal pattern was pronounced in agricultural streams with new ($F_{7,107} = 5.467$, $p = 0.003$) and no BMPs ($F_{7,107} = 2.241$, $p = 0.090$) (Table 2). NH_4^+ concentrations also increased in August and October, but this trend was more pronounced in agricultural streams as a whole than in reference streams. The increases in NH_4^+ coincided with decreases in NO_3^-. DOC followed a similar seasonal pattern, but these differences were not significant (Table 2). Peaks of nutrient concentrations occurred in reference streams but were not significantly different from baseline concentrations.

DA was used to assess how well stream category assignment corresponded with chemical and physical attributes of the streams. The analysis identified 3 main factors that explained a combined 69.84% of total variation. Overall, 51% of streams were correctly identified (Table 3), with reference streams being correctly identified in 81% of cases. All agricultural streams were correctly identified to their respective categories in only 40% of cases. The 2 factors that best predicted site assignment and that exposed the greatest differences across stream categories over the 3-y study were canopy cover and conductivity. The 3rd environmental factor affecting stream categorization was DOC, despite a lack of significant differences among stream category DOC content (Table 2).

Seasonal changes in algal taxonomic composition

Several seasonal changes in benthic algal taxonomic composition coincided with changes in FA composition. Diatoms were consistently the most abundant taxonomic algal group in all samples (Table 4). One exception was in autumn, when streams with no or new BMPs had significantly greater proportions of diatoms than did streams with old BMPs ($F_{3,28} = 10.338$, $p < 0.001$). Cyanobacterial taxa were least abundant in the spring months, but the proportion of cyanobacteria was significantly greater in reference streams than in streams with old BMPs ($F_{3,20} = 2.694$, $p = 0.074$). Several temporal patterns in specific algal taxa were also observed. The chantransia stage of a red alga (Rhodophyceae, possibly *Batrachospermum* sp.) was common during summer in reference streams and streams with old and new BMPs, but the gametophyte stage was not observed. Among reference streams, closely adhering monoraphid diatoms, including *Cocconeis pediculus* Ehrenb., *C. placentula* Ehrenb., and *Achnanthidium minutissimum* (Kütz.) Czarn, were most common. In contrast, biraphid diatoms, such as *Gomphonema augur* Ehrenb., and keeled diatoms, such as *Nitzschia palea* (Kütz.) W.Sm., were more common in agricultural streams. The mucilaginous colonial chrysophyte *Hydrurus foetidus* (Villars) Trevisan was present only in reference streams and strictly in spring when it could make up as much as 12% of the total taxa present in individual streams. A more detailed analysis of taxonomic composition of these assemblages is available elsewhere (Whorley and Wehr 2016).

Essential FA content in relation to BMP presence

Total FA content in benthic algal assemblages differed strongly and significantly among reference and agricultural stream categories ($F = 9.978$, $p < 0.001$; Fig. 3). Benthic algae in agricultural streams with no BMPs had ~2× the average total FA content (1.88 mg/m^2 more) than assemblages in reference streams (Tukey's HSD, $p < 0.001$). Benthic algae in agricultural streams with BMPs had intermediate total FA content that was 50% greater (1.02 mg/m^2 more) than FA content in algae from reference streams (Tukey's HSD, $p < 0.02$). Total FA content in benthic algae did not differ between streams with old or new BMPs (Tukey's HSD, $p = 0.987$). In all systems, total FA content was positively correlated with algal biomass and stoichiometric measurements, based on chlorophyll *a* ($r_s = 0.621$), algal N content ($r_s = 0.682$), and P content ($r_s = 0.715$) (Fig. 4A–C). These trends were consistent within stream categories (all $r_s > 0.400$).

Specific categories of FA compounds differed among stream categories, particularly with regard to total ω-3 (Σω3) FAs ($F = 5.961$, $p = 0.001$) and Σω6 ($F = 6.795$, $p < 0.001$) content (Fig. 3). Benthic algal Σω3 content averaged 0.53 mg/m^2 less (45%) in reference streams than in agricultural streams with old BMPs (Tukey's HSD, $p = 0.077$), and 0.98 mg/m^2 less (69%) than in agricultural streams lacking BMPs (Tukey's HSD, $p < 0.001$). However, average content of Σω3 FA in reference streams differed by only 0.40 mg/m^2 (36%) from that measured in algae colonizing streams with newly installed BMPs (Tukey's HSD, $p = 0.281$). Similar patterns were observed for Σω6 content with regard to stream categories. Quantities of the remaining benthic algal FA compounds (Σother) also differed among stream categories ($F = 10.669$, $p < 0.001$). Benthic algae averaged 0.61 mg/m^2 less in reference streams than in agricultural streams as a whole (Tukey's HSD, $p < 0.001$) but did not differ between agricultural streams with and without BMPs installed (Tukey's HSD, $p > 0.10$).

A PCA ordination was performed using monthly averages (total of 23 mo) of all structural groups of FAs measured in benthic algae (total FA, Σω3, Σω6, Σother) in each stream category, combined with corresponding physical and chemical characteristics of the streams (Fig. 5A). This analysis explained 45.82% of the total variation in the data set within the first 2 axes. Quantities of benthic algal FAs, across structural categories, were strongly inversely associated with canopy cover (greatest in reference streams), but only weakly with NO_3^-, NH_4^+, SRP, DOC, specific conductivity, pH, and TSS. The resulting NMDS plot of these relationships revealed a group composed primarily of ref-

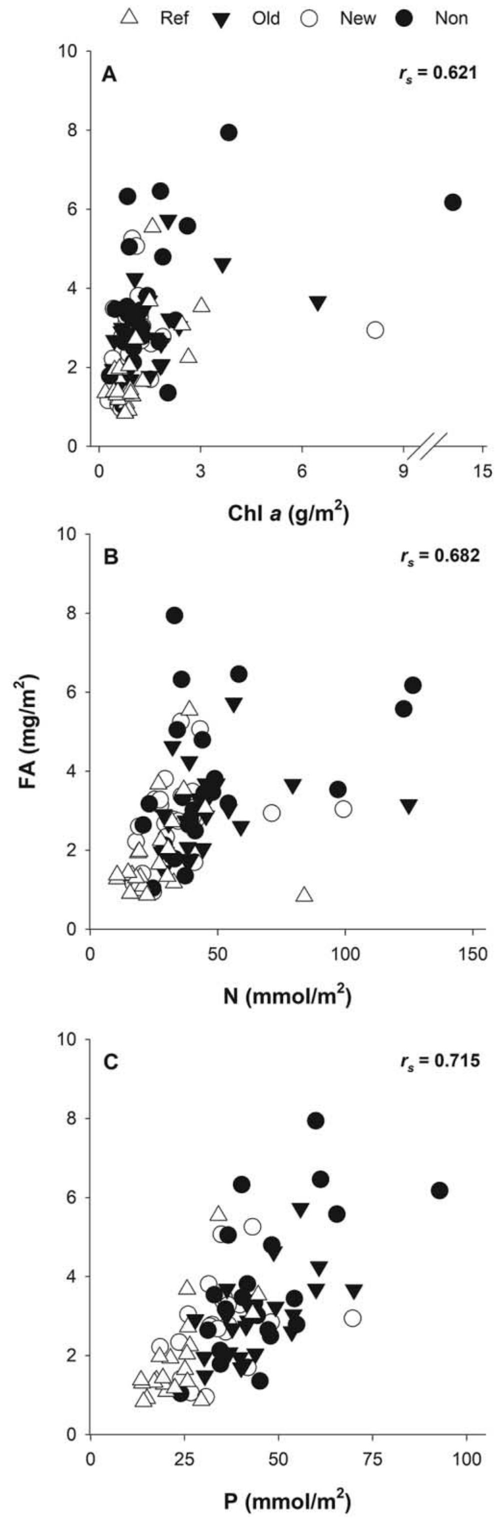

Figure 4. Scatterplot and Spearman's correlation coefficients for total benthic algal fatty-acid (FA) content and chlorophyll *a* (Chl *a*) (A), total N (B), and total P (C) content. See Fig. 2 for stream categories.

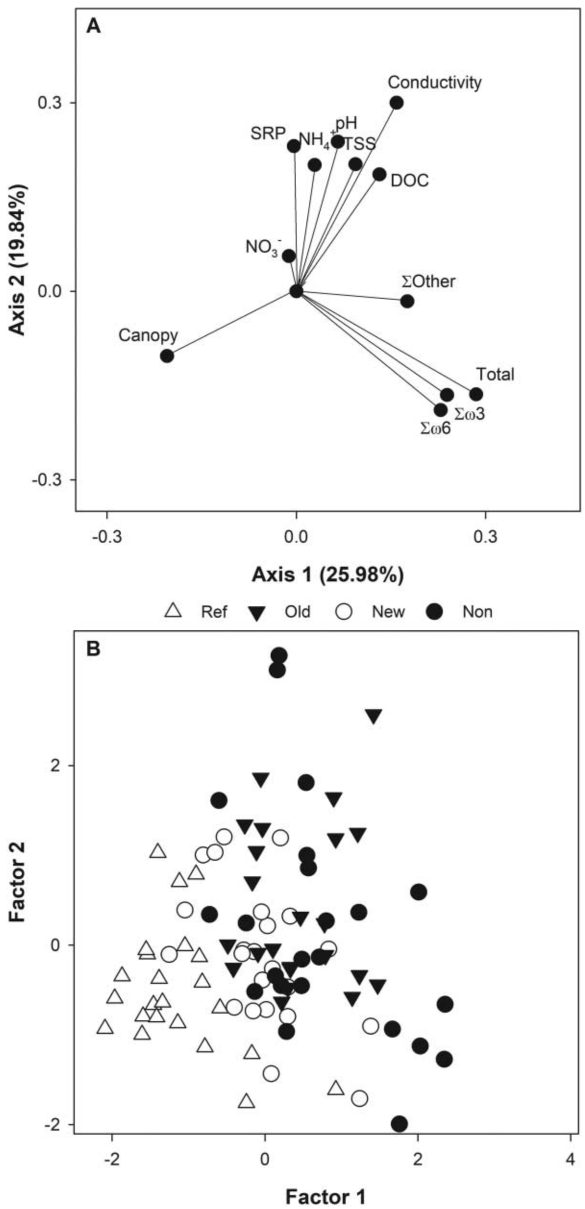

Figure 5. A.—Principal components analysis (PCA) of relationships among groups of fatty-acid (FA) compounds and stream chemistry and nutrient conditions, showing environmental variables averaged by category and year (45.82% of total variation summarized in first 2 axes). B.—Nonmetric multidimensional scaling (NMDS) ordination of streams shows relationships among stream categories and environmental variables. See Fig. 2 for stream categories.

erence streams that differed distinctly from agricultural stream categories (Fig. 5B).

Amounts of ω3 and ω6 FA compounds in benthic algae differed significantly among stream categories (Fig. 6). We compared 5 essential FAs: linoleic acid (LIN 18:2ω6), α-linolenic acid (ALA 18:3ω3), arachidonic acid (ARA 20:4ω6), eicosapentaenoic acid (EPA 20:5ω3), and docosahexaenoic acid (DHA 22:6ω3). Agricultural streams had 44% greater content of ALA ($F_{3,430}$= 5.582, p = 0.001) and 38% greater content of LIN ($F_{3,430}$= 6.761, $p < 0.001$) than did reference streams (Tukey's HSD, $p < 0.10$). EPA content varied among agricultural stream categories ($F_{3,430}$= 4.153, p = 0.006). Algae in streams lacking BMPs had 0.499 mg/m^2 (Tukey's HSD, p = 0.053) greater concentrations than algae in streams with new BMPs and 0.660 mg/m^2 more EPA than algae in reference streams (Tukey's HSD, p = 0.004). Content of ARA also varied among stream categories ($F_{3,430}$ = 2.607, p = 0.051). Algae in agricultural streams lacking BMPs had 0.046 mg/m^2 (Tukey's HSD, p = 0.051) greater content than algae in streams with new BMPs and 0.042 mg/m^2 more EPA than algae in reference streams (Tukey's HSD, p = 0.081). Agricultural streams with no BMPs also had 58% more DHA ($F_{3,430}$= 7.897, $p < 0.001$) than algae in all other stream categories (Tukey's HSD, $p < 0.10$).

DA complemented these results (Table 3). This analysis, which included the amounts of all individual compounds measured (Table S1), identified 3 main factors that explained only 26.61% of total variation. Overall 44% of streams were correctly identified, and reference streams were identified correctly in 76% of cases. All agricultural streams were identified correctly in only 33% of cases. The measurements that provided the greatest predictability of site assignment were DHA and ARA content. Oleic acid (18:1ω9) also was a reliable predictor. Algal assemblages in agricultural streams on average had 43% more oleic acid ($F_{3,430}$ = 11.510, $p < 0.001$) than algae in reference streams (Tukey's HSD, $p < 0.10$).

Next, we examined the relationships among stream physical and chemical characteristics and key FA compounds

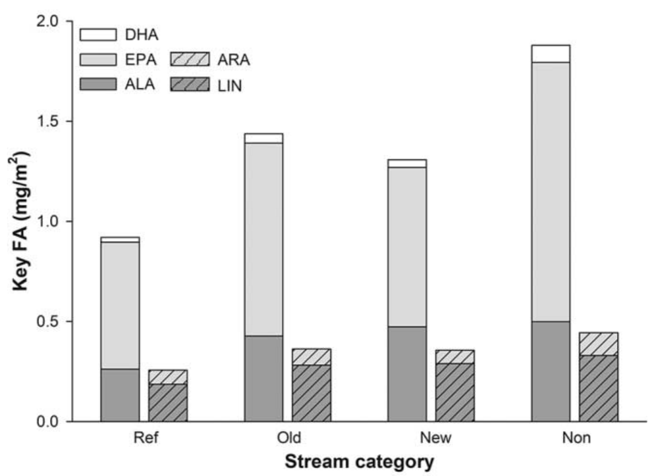

Figure 6. Essential fatty-acid (FA) compound amounts (mg/m^2) by stream category, averaged over the course of the entire study. Compounds included are α-linolenic acid (ALA 18:3ω3), eicosapentaenoic acid (EPA 20:5ω3), docosahexaenoic acid (DHA 22:6ω3), linoleic acid (LIN 18:2ω6), and arachidonic acid (ARA 20:4ω6). Open bars are ω3 compounds, and hatched bars are ω6 compounds.

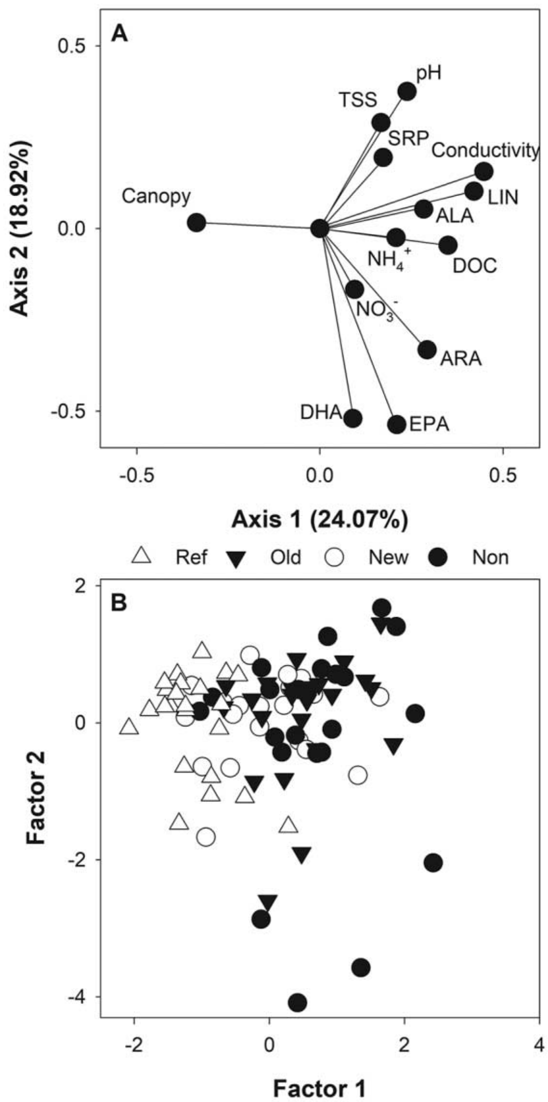

Figure 7. A.—Principal components analysis (PCA) of relationships among essential fatty-acid (FA) compounds and stream chemistry and nutrient conditions, showing environmental variables averaged by category and year (24.07 and 18.92% of total variation summarized in first 2 axes). B.—Nonmetric multidimensional scaling (NMDS) ordination of streams shows relationships among stream categories and environmental variables. See Fig. 2 for stream categories.

(Fig. 7A). As with the broad structural categories, canopy cover was strongly inversely associated with all essential FA compounds. ALA and LIN were most closely associated with SRP and conductivity, whereas EPA, DHA, and ARA were more closely associated with aqueous NO_3^-. Nevertheless, <43% of the variation among these variables was explained by this relationship. Reference streams contained lower amounts of these individual compounds but the NMDS did not separate the 4 agricultural stream categories clearly (Fig. 7B).

Seasonal variation in algal essential FAs

The amounts of the main FA categories increased in October–November but were fairly stable from April to September (Table 5). Benthic algal FA content differed significantly among months across all FA categories (total: $F_{3,53}$= 8.003, p < 0.001; Σω3: $F_{3,53}$ = 13.385, p < 0.001; Σω6: $F_{3,53}$ = 9.048, p < 0.001; Σothers, $F_{3,53}$ = 2.287, p = 0.027). Total FA content increased most (1.75 mg/m^2; 58%) from September to October (Tukey's HSD, p = 0.011), as did amounts of Σω3 and Σω6. Essential FA compounds varied seasonally (Table 6). LIN ($F_{3,53}$ = 2.127, p = 0.054), ARA ($F_{3,53}$ = 7.192, p < 0.001), EPA ($F_{3,53}$ = 16.080, p < 0.001), and DHA ($F_{3,53}$ = 5.645, p < 0.001) all differed significantly among months. LIN varied significantly between April and August (+0.168 mg/m^2), but levels of ARA (+0.117 mg/m^2), EPA (+1.551 mg/m^2), and DHA (+0.085 mg/m^2) were greatest in October and November. In contrast, levels of ALA did not vary markedly over the year ($F_{3,53}$ = 1.358, p = 0.240).

Over the 3 y of our study, differences in benthic algal FA content among the different BMP treatments were most pronounced in summer (June–August; Table 5). No statistically significant differences were observed among the stream categories in April–May or October–November. However, from June through August, benthic algae Σω3 and Σω6 FA content changed significantly among stream categories. Essential FA compounds exhibited similar differences over the 3 y. Relationships between benthic algal essential FA content and agricultural BMPs were most pronounced during June, July, and August (Table 6). The greatest differences were observed in June where reference streams had significantly less ALA ($F_{3,53}$= 2.388, p = 0.079), DHA ($F_{3,53}$= 3.376, p = 0.025), and ARA ($F_{3,53}$= 2.594, p = 0.062) than ≥1 agricultural stream category. Reference stream benthic algal assemblages had significantly less ALA ($F_{3,34}$ = 2.616, p = 0.067) and DHA ($F_{3,34}$ = 2.282, p = 0.097) than did algae in agricultural streams. In contrast, quantities of EPA did not differ among stream categories in any month. Benthic algal FA content differed strongly interannually (Figs 8A, B, 9A, B). For example, total FA amounts were ~1.4 mg/m^2 lower in reference streams, ~1.7 mg/m^2 lower in BMP streams, and ~3.8 mg/m^2 than in agricultural streams without BMPs in year 2 than year 3. Σω6 (F = 6.685, p = 0.001) also differed among years, where amounts during October and November in year 2 decreased compared to the summer, whereas during the other years, amounts increased during these months.

DISCUSSION

Upper Delaware streams receive limited agricultural effects

We observed statistically significant differences in water chemistry and other ecological variables between reference and agriculturally affected streams in Upper Delaware

Table 5. Analysis of variance and Tukey's Honest Significant Difference (HSD; $p < 0.1$) comparing mean (SE) monthly fatty-acid (FA; mg/m^2) types among stream categories. n/a = no differences. See Table 2 for category abbreviations.

Month		Total	Σω3	Σω6	Σother
April	Ref	1.3 (0.2)	0.5 (0.1)	0.3 (0.1)	0.5 (0.1)
	Old	1.8 (0.3)	0.7 (0.2)	0.3 (0.04)	0.9 (0.1)
	New	1.2 (0.2)	0.4 (0.1)	0.2 (0.03)	0.6 (0.1)
	Non	2.7 (1.0)	1.2 (0.7)	0.4 (0.1)	1.1 (0.3)
	$F_{3,53}$ (p)	1.969 (0.130)	1.407 (0.251)	1.496 (0.226)	2.434 **(0.075)**
	Differences	n/a	n/a	n/a	Ref < Non
May	Ref	1.7 (0.3)	0.6 (0.1)	0.4 (0.1)	0.7 (0.1)
	Old	2.8 (0.5)	1.0 (0.3)	0.5 (0.1)	1.4 (0.3)
	New	2.6 (0.7)	1.0 (0.4)	0.4 (0.1)	1.2 (0.5)
	Non	3.2 (0.8)	1.0 (0.2)	0.4 (0.1)	1.7 (0.6)
	$F_{3,53}$ (p)	1.121 (0.349)	0.649 (0.587)	0.169 (0.917)	1.398 (0.254)
	Differences	n/a	n/a	n/a	n/a
June	Ref	1.0 (0.2)	0.4 (0.1)	0.2 (0.04)	0.4 (0.1)
	Old	2.8 (0.5)	1.1 (0.3)	0.5 (0.1)	1.2 (0.3)
	New	3.4 (0.9)	1.6 (0.6)	0.6 (0.1)	1.3 (0.3)
	Non	3.3 (0.5)	1.5 (0.4)	0.5 (0.1)	1.3 (0.3)
	$F_{3,53}$ (p)	**3.837 (0.015)**	2.072 (0.115)	**3.248 (0.029)**	**3.168 (0.032)**
	Differences	Ref < All Ag	n/a	Ref < New, Non	Ref < All Ag
July	Ref	1.4 (0.1)	0.5 (0.1)	0.4 (0.1)	0.4 (0.04)
	Old	2.6 (0.6)	1.1 (0.4)	0.7 (0.1)	0.8 (0.1)
	New	2.4 (0.4)	1.1 (0.3)	0.1 (0.1)	0.8 (0.1)
	Non	3.0 (0.3)	1.1 (0.1)	0.8 (0.1)	1.0 (0.1)
	$F_{3,53}$ (p)	**2.910 (0.043)**	1.494 (0.227)	**3.317 (0.027)**	**4.79 (0.005)**
	Differences	Ref < Old, New	n/a	Ref < Old, New	Ref < All Ag
August	Ref	1.5 (0.1)	0.6 (0.1)	0.4 (0.04)	0.5 (0.1)
	Old	2.9 (0.5)	1.3 (0.3)	0.8 (0.1)	0.9 (0.1)
	New	3.3 (0.6)	1.4 (0.3)	0.8 (0.1)	1.1 (02)
	Non	3.4 (0.6)	1.4 (0.3)	0.9 (0.2)	1.1 (0.1)
	$F_{3,53}$ (p)	**3.239 (0.029)**	**2.199 (0.099)**	**3.016 (0.038)**	**5.531 (0.002)**
	Differences	Ref < New, Non	Ref < New	Ref < New, Non	Ref < New, Non
September	Ref	1.3 (0.2)	0.6 (0.1)	0.3 (0.04)	0.4 (0.1)
	Old	2.1 (0.3)	1.0 (0.2)	0.4 (0.1)	0.7 (0.1)
	New	1.8 (0.7)	0.8 (0.3)	0.3 (0.1)	0.8 (0.3)
	Non	3.2 (1.0)	1.5 (0.5)	0.7 (0.3)	1.0 (0.3)
	$F_{3,34}$ (p)	1.956 (0.139)	1.921 (0.145)	2.062 (0.124)	1.545 (0.221)
	Differences	n/a	n/a	n/a	n/a
October	Ref	2.9 (0.7)	1.7 (0.4)	0.7 (0.1)	0.6 (0.1)
	Old	4.0 (0.8)	2.4 (0.6)	0.7 (0.1)	0.9 (0.1)
	New	3.6 (0.7)	2.0 (0.4)	0.6 (0.1)	1.0 (0.2)
	Non	5.1 (1.3)	3.7 (0.9)	1.0 (0.2)	1.0 (0.2)
	$F_{3,53}$ (p)	1.110 (0.353)	1.025 (0.389)	1.281 (0.290)	1.468 (0.234)
	Differences	n/a	n/a	n/a	n/a
November	Ref	4.0 (0.8)	2.4 (0.5)	0.8 (0.2)	0.9 (0.2)
	Old	4.4 (0.9)	2.9 (0.7)	0.7 (0.1)	0.8 (0.1)
	New	3.5 (0.7)	2.0 (0.5)	0.7 (0.1)	0.9 (0.2)
	Non	6.0 (1.0)	4.0 (0.9)	0.9 (0.2)	1.0 (0.1)
	$F_{3,53}$ (p)	1.350 (0.268)	1.921 (0.145)	0.585 (0.627)	0.268 (0.848)
	Differences	n/a	n/a	n/a	n/a

Table 6. Analysis of variance and Tukey's Honest Significant Difference (HSD; $p < 0.1$) comparing mean (SE) monthly essential fatty-acid (FA; mg/m^2) types among stream categories. n/a = no differences. See Table 2 for category abbreviations.

Month	Category	ALA (18:3ω3)	EPA (20:5ω3)	DHA (22:6ω3)	LIN (18:2ω6)	ARA (20:4ω6)
April	Ref	0.4 (0.1)	0.2 (0.04)	0.1 (0.01)	0.2 (0.03)	0.02 (0.01)
	Old	0.4 (0.1)	0.3 (0.1)	0.03 (0.01)	0.2 (0.03)	0.03 (0.01)
	New	0.3 (0.1)	0.1 (0.03)	0.02 (0.01)	0.1 (0.02)	0.01 (0.01)
	Non	0.5 (0.2)	0.7 (0.5)	0.04 (0.02)	0.3 (0.1)	0.04 (0.01)
	$F_{3,53}$ (p)	0.965 (0.416)	1.431 (0.244)	2.045 (0.119)	1.287 (0.289)	1.748 (0.168)
	Differences	n/a	n/a	n/a	n/a	n/a
May	Ref	0.4 (0.1)	0.2 (0.1)	0.02 (0.01)	0.2 (0.1)	0.03 (0.01)
	Old	0.6 (0.2)	0.3 (0.1)	0.03 (0.01)	0.3 (0.1)	0.1 (0.02)
	New	0.5 (0.1)	0.4 (0.3)	0.02 (0.01)	0.3 (0.1)	0.1 (0.03)
	Non	0.6 (0.1)	0.4 (0.1)	0.1 (0.02)	0.3 (0.1)	0.1 (0.02)
	$F_{3,53}$ (p)	0.584 (0.628)	0.628 (0.600)	1.573 (0.207)	0.282 (0.838)	0.843 (0.476)
	Differences	n/a	n/a	n/a	n/a	n/a
June	Ref	0.3 (0.1)	0.1 (0.1)	0.01 (0.01)	0.1 (0.02)	0.02 (0.01)
	Old	0.6 (0.1)	0.5 (0.2)	0.04 (0.01)	0.3 (0.1)	0.1 (0.03)
	New	0.9 (0.3)	0.6 (0.3)	0.03 (0.02)	0.4 (0.1)	0.1 (0.02)
	Non	0.6 (0.1)	0.8 (0.3)	0.1 (0.02)	0.3 (0.1)	0.1 (0.01)
	$F_{3,53}$ (p)	**2.388 (0.079)**	1.338 (0.272)	**3.376 (0.025)**	**3.423 (0.024)**	**2.594 (0.062)**
	Differences	Ref < New	n/a	Ref < Non	Ref < New	Ref < Old
July	Ref	0.1 (0.03)	0.4 (0.1)	0.02 (0.01)	0.2 (0.04)	0.04 (0.01)
	Old	0.4 (0.2)	0.7 (0.3)	0.03 (0.01)	0.4 (0.1)	0.1 (0.02)
	New	0.4 (0.1)	0.7 (0.2)	0.02 (0.01)	0.4 (0.1)	0.1 (0.02)
	Non	0.4 (0.1)	0.7 (0.1)	0.03 (0.01)	0.4 (0.04)	0.1 (0.01)
	$F_{3,53}$ (p)	0.918 (0.439)	0.902 (0.446)	0.389 (0.764)	**3.255 (0.029)**	1.040 (0.383)
	Differences	n/a	n/a	n/a	Ref < Non	n/a
August	Ref	0.1 (0.03)	0.4 (0.1)	0.02 (0.01)	0.2 (0.02)	0.04 (0.01)
	Old	0.3 (0.2)	0.9 (0.2)	0.03 (0.01)	0.4 (0.1)	0.1 (0.02)
	New	0.5 (0.1)	1.0 (0.2)	0.02 (0.01)	0.5 (0.1)	0.1 (0.04)
	Non	0.5 (0.2)	0.9 (0.2)	0.02 (0.01)	0.4 (0.1)	0.1 (0.1)
	$F_{3,53}$ (p)	1.717 (0.175)	1.947 (0.133)	0.461 (0.710)	**4.024 (0.012)**	1.383 (0.258)
	Differences	n/a	n/a	n/a	Ref < New, Non	n/a
September	Ref	0.2 (0.1)	0.4 (0.1)	0.01 (0.01)	0.1 (0.02)	0.1 (0.01)
	Old	0.4 (0.1)	0.6 (0.2)	0.02 (0.01)	0.2 (0.04)	0.1 (0.01)
	New	0.3 (0.1)	0.5 (0.2)	0.01 (0.01)	0.2 (0.1)	0.1 (0.02)
	Non	0.7 (0.2)	0.8 (0.3)	0.03 (0.01)	0.1 (0.2)	0.2 (0.06)
	$F_{3,34}$ (p)	**2.616 (0.067)**	0.891 (0.456)	**2.282 (0.097)**	2.075 (0.123)	1.867 (0.154)
	Differences	Ref < Non	n/a	Ref < Non	n/a	n/a
October	Ref	0.2 (0.1)	1.5 (0.4)	0.1 (0.02)	0.2 (0.04)	0.2 (0.1)
	Old	0.4 (0.1)	1.9 (0.6)	0.1 (0.1)	0.2 (0.1)	0.2 (0.1)
	New	0.5 (0.1)	1.5 (0.3)	0.1 (0.03)	0.3 (0.1)	0.1 (0.03)
	Non	0.3 (0.1)	2.6 (0.9)	0.2 (0.1)	0.3 (0.1)	0.3 (0.10)
	$F_{3,53}$ (p)	1.329 (0.275)	0.944 (0.426)	2.115 (0.109)	0.381 (0.767)	1.019 (0.392)
	Differences	n/a	n/a	n/a	n/a	n/a
November	Ref	0.4 (0.1)	1.9 (0.5)	0.1 (0.04)	0.3 (0.1)	0.2 (0.1)
	Old	0.3 (0.1)	2.4 (0.6)	0.1 (0.03)	0.2 (0.1)	0.1 (0.02)
	New	0.4 (0.1)	1.5 (0.4)	0.1 (0.03)	0.3 (0.1)	0.1 (0.02)
	Non	0.5 (0.1)	3.3 (0.8)	0.2 (0.1)	0.3 (0.1)	0.1 (0.02)
	$F_{3,53}$ (p)	0.341 (0.796)	1.926 (0.137)	**3.289 (0.028)**	0.251 (0.860)	1.414 (0.249)
	Differences	n/a	n/a	Ref, New < Non	n/a	n/a

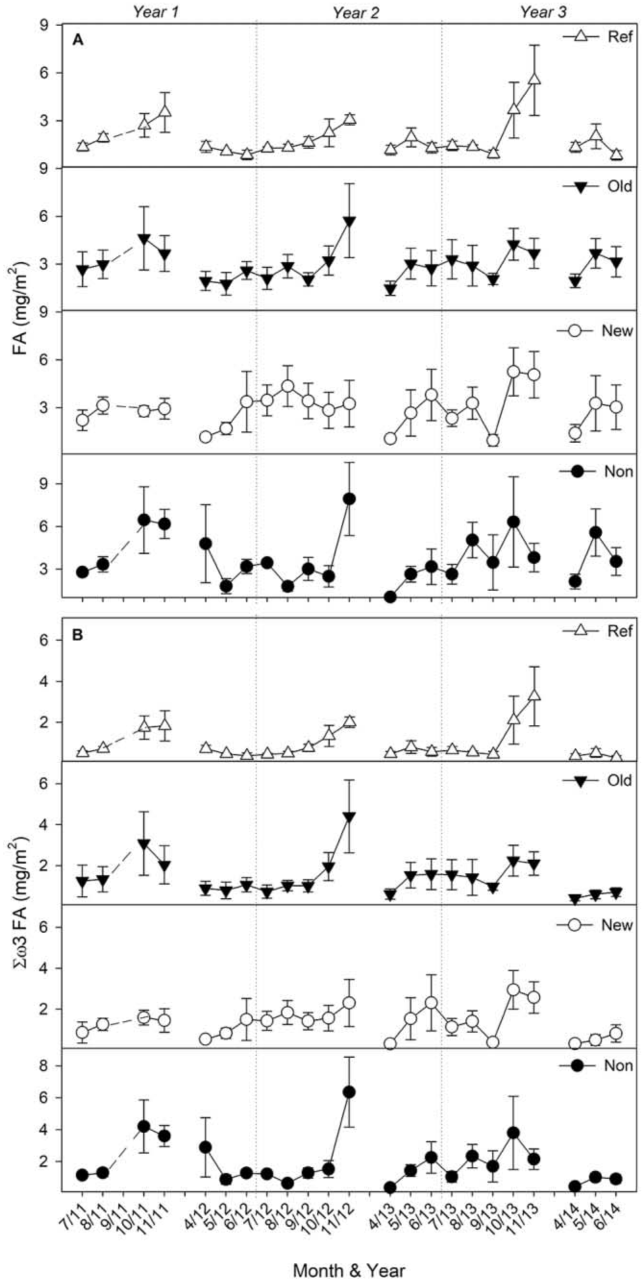

Figure 8. Mean (±SE) monthly total fatty acids (FAs) (A) and Σω3 FAs (B). See Fig. 2 for stream categories.

streams. In general, streams draining agricultural lands and lacking BMPs did have elevated inorganic N concentrations (as NO_3^-, NH_4^+) relative to reference streams. However elevated, N concentrations and many other variables, including SRP, conductivity, dissolved O_2, pH, and turbidity, fell within widely used water-quality recommendations (NYSDEC 1999, USEPA 2001, Dodds and Oakes 2004). Nutrient reduction is a central goal for stream-based BMPs. By this criterion, the overall quality of these streams may be regarded as only modestly affected by agriculture. Nevertheless, these differences among stream-management categories affect local algal production (Stevenson et al. 1996, Mulbury et al. 2008). We demonstrated they also affect algal biochemical composition, which may have important implications for aquatic food webs and ecosystem integrity.

Within each season, the relative proportions of major algal groups differed noticeably among stream categories and seasons, but nearly all algal assemblages were dominated by

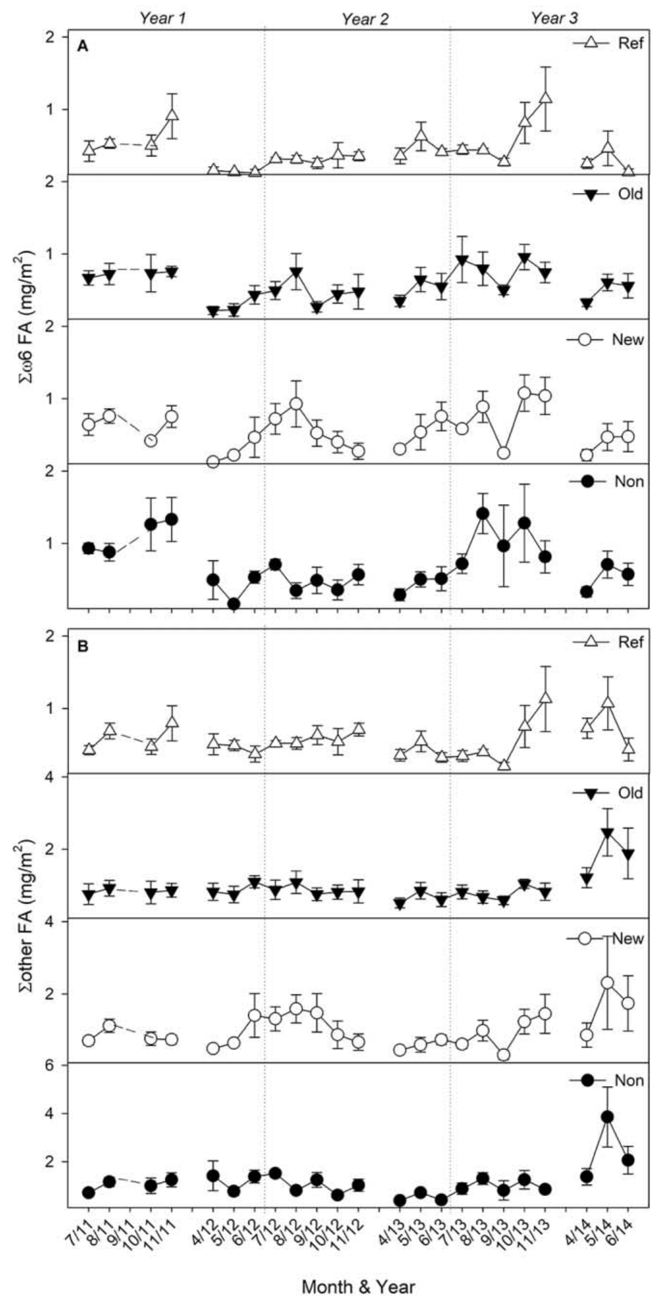

Figure 9. Mean (±SE) monthly Σω6 fatty acids (FAs) (A) and Σother FAs (B). See Fig. 2 for stream categories.

diatom taxa. Common diatom taxa in reference streams were prostrate monoraphid taxa, whereas biraphid and keeled diatom taxa were more likely to be in agricultural streams. This finding is in keeping with previously observed records of motile diatom taxa in nutrient-rich environments (Pringle et al. 1988, Pringle 1990). Of special note is the prevalence of the benthic chrysophyte *Hydrurus foetidus* in reference streams. *Hydrurus foetidus* is a pollution-sensitive taxon not found in habitats or seasons where water temperature is >16°C and is sensitive to a variety of environmental disturbances (Klaveness 2017 and references therein). It is also a prolific producer of PUFA compounds, including EPA and DHA (Klaveness 2017). Despite the lack of significant impairment of agricultural streams, the lack of *Hydrurus* in those streams compared with its presence in reference streams indicates the sensitive nature of algal assemblages to environmental disturbances. Further discussion of patterns and changes in algal taxonomic changes in relationships to agricultural disturbance has been presented recently (Whorley and Wehr 2016).

Agriculture and BMP effects on benthic algal FAs

We are among the first investigators to examine the effects of BMPs on longer-term trends in biochemical properties of stream primary producers and their relationship to physical and chemical properties of streams in any landscape. Our data demonstrate that FA concentrations are consistently (3-y duration) and significantly lower in benthic algae in reference than in agricultural streams. Moreover, among agricultural streams with different management conditions, total FA concentrations in algae were lower in streams with than without BMPs. Despite frequent sampling over 3 y, we did not detect a difference between streams with new (≤2 y at start of study) and old (≥3 y) BMPs. We interpret this result to mean that the effect of BMPs on the nutritional quality of basal food sources is fairly immediate and sustained over multiple years.

Our data also suggest that the influence of agriculture practices on benthic algal assemblages, through changes in biomass and FA production, is mediated primarily through altered light levels, and then by greater nutrient inputs. Several investigators have observed a positive relationship between increasing algal biomass and increasing FA content in various aquatic ecosystems (Ziegler and Lyon 2010, Hill et al. 2011, Cashman et al. 2013, Guo et al. 2015). The streams we studied differed with regard to canopy cover, where agriculturally affected streams had significantly less streamside shading than did reference streams. Light availability can directly alter the production of both algal biomass and FAs (Guo et al. 2015). We did not test directly the effects of canopy cover on FA production, but this influence does contribute to benthic algal FA differences, especially when examined in conjunction with nutrient addition (Cashman et al. 2013). Ziegler and Lyon (2010) observed nearly a doubling of FA concentration (expressed as μg FA/g C) in response to increasing stream N, P, and C content in an agricultural watershed. Our data corroborate these findings for biomass and stoichiometry.

Our data add support to the idea that analyzing biomass or C alone may not provide a complete picture of the effects that environmental stressors may have on benthic algal assemblages and the food webs they support (Brett et al. 2017). Benthic algal assemblages in agricultural streams in our study contained greater amounts of essential ω3 (ALA, EPA, DHA) and ω6 (LIN, ARA) molecules than did algal assemblages in reference streams. Notably, agricultural stream algae also contained greater amounts of oleic acid (18:1ω9), which is commonly associated with animal feces (Williams et al. 1960). The effects of elevated nutrients and altered light regimes on FA composition are not always straightforward. Experiments in which light and nutrient availability were manipulated demonstrated that benthic algal ALA increased with greater light availability, DHA decreased with elevated nutrients, and EPA decreased with excess light and nutrients (Cashman et al. 2013). An observational study of phytoplankton FA concentrations found that phytoplankton produced greater concentrations of essential FA compounds (e.g., EPA, DHA) during periods with lower nutrient concentrations and fewer cyanobacterial taxa compared to periods of time with higher nutrient concentrations and greater proportions of cyanobacterial taxa (Galloway and Winder 2015). However, these investigators examined the effect of relatively extreme nutrient-enrichment conditions. The elevated nutrient concentrations observed in our study were moderate by comparison.

The moderate intensity of agricultural activity in the Upper Delaware may have led to greater nutritional quality in benthic food resources, but further human disturbance could be detrimental to algal lipid profiles. Several classes of FAs decrease in algal assemblages under more severe eutrophication (especially increasing SRP and DIN), coupled with other factors associated with urbanization (Boëchat et al. 2014). In the Esopus River system (New York, USA), the combined effects of increased sedimentation and nutrients downstream of a reservoir resulted in reduced concentrations of ARA, EPA, and DHA in algal assemblages (George et al. 2016). FA profiles respond to low as well as extreme levels of disturbance, so they highlight the more complex effects such alterations have on riverine food webs and may be useful in tracking the progress of restoration practices.

Seasonal variations in algal essential FAs

Few investigators thus far have quantified benthic algal FA trends over extended periods of time. We identified a key pattern across 3 y and among diverse stream types: a consistent increase in FA content in autumn. The factors creating this trend are not certain, but may be associated with increasing light availability (reduced canopy cover) and nutrient inputs from farm runoff and autumn-shed decaying leaf litter. In our study, increases in FA content also varied in their magnitude with differences in broader climactic events. The autumnal increase in benthic algal FA in 2012, a drought year, was 42% less than in 2013, when flows were near normal (Artusa 2015). Our data suggest that assemblages in reference streams were most sensitive to this climactic abnormality. Nearly all of the essential FAs measured followed this seasonal trend, with the exception of α-linolenic acid (ALA), which is a central precursor (Desvilettes and Bec 2009) to critical ω3 compounds EPA and DHA. Recent analyses of algal lipid data from global sites has revealed that warming temperatures, which often accompany drought conditions, can lead to a decrease in algal nutritional quality, especially in concentrations of metabolically important ω3 FAs (Hixson and Arts 2016). In a study of Appalachian headwater streams in Pennsylvania, Honeyfield and Maloney (2014) also reported seasonal differences in benthic algal FAs, which they attributed to changes in temperature, canopy cover, and conductivity, although patterns were not statistically significant.

FA profiles and concentrations are increasingly important tools for bioassessment. Understanding the effects of disturbance and mitigation on biotic assemblages within the context of ecosystem services can be a powerful tool for bioassessment efforts that extend beyond taxonomic composition and diversity. Our findings suggest that changes in the biochemical properties of basal food resources (benthic algae) are sensitive indicators of ecological change and reflect important foodweb effects that data on species composition may not reflect. Specifically, bioassessment data sets based on algal FA profiles can provide important biochemical information on the health of stream ecosystems, data which may be more sensitive to temporal and human disturbance gradients than species composition.

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LITERATURE CITED

Allan, J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35:257–284.

Artusa, A. 2015. United States drought monitor. National Drought Mitigation Center (NDMC), University of Nebraska–Lincoln, Lincoln, Nebraska. (Available from: http://droughtmonitor.unl.edu/)

Bishop, P. L., W. D. Hively, J. R. Stedinger, M. R. Rafferty, J. L. Lojpersberger, and J. A. Bloomfield. 2005. Multivariate analysis of paired watershed data to evaluate agricultural best management practice effects on stream water phosphorus. Journal of Environmental Quality 34:1087–1101.

Boëchat, I. G., A. Krüger, R. C. Chavesa, D. Graeberd, and B. Gückera. 2014. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. Science of the Total Environment 482:62–70.

Boëchat, I. G., A. Krüger, A. Giani, C. C. Figueredo, and B. Gücker. 2011. Agricultural land-use affects the nutritional quality of stream microbial communities. FEMS Microbiology Ecology 77:568–576.

Bosch, I., J. C. Makarewicz, T. W. Lewis, E. A. Bonk, M. Finiguerra, and B. Groveman. 2009. Management of agricultural practices results in declines of filamentous algae in the lake littoral. Journal of Great Lakes Research 35:90–98.

Brannan, K. M., S. Mostaghimi, P. W. McClellan, and S. Inamdar. 2000. Animal waste BMP impacts on sediment and nutrient losses in runoff from the Owl Run watershed. Transactions of the American Society of Agricultural Engineers 43:1155–1166.

Brett, M. T., S. E. Bunn, S. Chandra, A. W. E. Galloway, F. Guo, M. J. Kainz, P. Kankaala, D. C. P. Lau, T. P. Moulton, M. E. Power, J. B. Rasmussen, S. J. Taipale, J. H. Thorp, and J. D. Wehr. 2017. How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? Freshwater Biology 62:833–853.

Brett, M., and D. Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483–499.

Carr, G. M., A. Morin, and P. A. Chambers. 2005. Bacteria and algae in stream periphyton along a nutrient gradient. Freshwater Biology 50:1337–1350.

Cashman, M. J., J. D. Wehr, and K. Truhn. 2013. Elevated light and nutrients alter the nutritional quality of stream periphyton. Freshwater Biology 58:1447–1457.

Desvilettes, C., and A. Bec. 2009. Formation and transfer of fatty acids in aquatic microbial food webs: role of heterotrophic protists. Pages 25–42 *in* M. T. Arts, M. T. Brett, and M. J. Kainz (editors). Lipids in aquatic ecosystems. Springer, New York.

Dodds, W. K., and R. M. Oakes. 2004. A technique for establishing reference nutrient concentrations across watersheds affected by humans. Limnology and Oceanography: Methods 2:333–341.

Dodds, W. K., and E. B. Welch. 2000. Establishing nutrient criteria in streams. Journal of the North American Benthological Society 19:186–196.

Ehrman, T. P., and G. A. Lamberti. 1992. Hydraulic and particulate matter retention in a 3rd-order Indiana stream. Journal of the North American Benthological Society 11:341–349.

Frost, P. C., R. S. Stelzer, G. A. Lamberti, and J. J. Elser. 2002. Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C : N : P ratios in lentic and lotic habitats. Journal of the North American Benthological Society 21:515–528.

Gabel, K. W., J. D. Wehr, and K. M. Truhn. 2012. Assessment of the effectiveness of best management practices for streams draining agricultural landscapes using diatoms and macroinvertebrates. Hydrobiologia 680:247–264.

Galloway, A. W., and M. Winder. 2015. Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. PLoS ONE 10:e0130053.

George, S. D., A. G. Ernst, B. P. Baldigo, and D. C. Honeyfield. 2016. Response of periphyton fatty acid composition to supplemental flows in the upper Esopus Creek, Catskill Mountains, New York (No. 2015-5161). US Geological Survey, Reston, Virginia.

Gitau, M. W., W. J. Gburek, and P. L. Bishop. 2008. Use of the SWAT model to quantify water quality effects of agricultural BMPs at the farm-scale. Transactions of the American Society of Agricultural and Biological Engineers 51:1925–1936.

Grolleau, G., and L. M. J. McCann. 2012. Designing watershed programs to pay farmers for water quality services: case studies of Munich and New York City. Ecological Economics 76:87–94.

Guo, F., M. J. Kainz, F. Sheldon, and S. E. Bunn. 2015. Spatial variation in periphyton fatty acid composition in subtropical streams. Freshwater Biology 60:1411–1422.

Hill, W. R., J. Rinchard, and S. Czesny. 2011. Light, nutrients and the fatty acid composition of stream periphyton. Freshwater Biology 56:1825–1836.

Hixson, S. M., and M. T. Arts. 2016. Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. Global Change Biology 22:2744–2755.

Honeyfield, D. C., and K. O. Maloney. 2014. Seasonal patterns in stream periphyton fatty acids and community benthic algal composition at six high-quality headwater streams. Hydrobiologia 744:35–47.

Ice, G. 2004. History of innovative best management practice development and its role in addressing water quality limited waterbodies. Journal of Environmental Engineering ASCE 130: 684–689.

Jack, J., R. H. Kelley, and D. Stiles. 2006. Using stream bioassessment protocols to monitor impacts of a confined swine operation. Journal of the American Water Resources Association 42:747–753.

Jeffrey, S. W., and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, and *c* in higher plants, algae, and natural phytoplankton. Biochemie und Physiologie der Pflanzen 167:191–194.

Klaveness, D. 2017. *Hydrurus foetidus* (Chrysophyceae)—an inland macroalga with potential. Journal of Applied Phycology 29:1485–1491.

Lamberti, G. A. 1996. The role of periphyton in benthic food webs. Pages 533–572 *in* R. J. Stevenson, M. Bothwell, and R. L. Lowe (editors). Algal ecology. Academic Press, Los Angeles, California.

Larson, J. H., W. B. Richardson, B. C. Knights, L. A. Bartsch, M. R. Bartsch, J. C. Nelson, J. A. Veldboom, and J. M. Vallazza. 2013. Fatty acid composition at the base of aquatic food webs is influenced by habitat type and watershed land use. PloS ONE 8:e70666.

Lorenzen, C. J. 1967. Determination of chlorophyll and phaeopigments: Spectrophotometric equations. Limnology and Oceanography 12:343–346.

McNeely, C., J. F. Finlay, and M. E. Power. 2007. Grazer traits, competition, and carbon sources to a headwater stream food web. Ecology 88:391–401.

Mehaffey, M. H., M. S. Nash, T. G. Wade, D. W. Ebert, K. B. Jones, and A. Rager. 2005. Linking land cover and water quality in New York City's water supply watersheds. Environmental Monitoring and Assessment 107:29–44.

Mulbury, W., S. Kondrad, and J. Buyer. 2008. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. Journal of Applied Phycology 20:1079–1085.

Napolitano, G. E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems. Pages 21–44 *in* M. T. Arts and B. C. Wainman (editors). Lipids in freshwater ecosystems. Springer, New York.

Nerbonne, B. A., and B. Vondracek. 2001. Effects of local land use on physical habitat, benthic macroinvertebrates, and fish in the Whitewater River, Minnesota, USA. Environmental Management 28:87–99.

NYSDEC (New York State Department of Environmental Conservation). 1999. Surface water and groundwater quality standards and groundwater effluent limitations. Environmental Conservation Law §§ 3-0301[2][m], 15-0313, 17-0301, 17-0809.

Olsen, Y. 1999. Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture. Pages 161–202 *in* M. T. Arts and B. C. Wainman (editors). Lipids in freshwater ecosystems. Springer, New York.

Osborne, L. L., and D. A. Kovacic. 1993. Riparian vegetated buffer strips in water quality restoration and stream management. Freshwater Biology 29:243–258.

Parrish, C. C. 1999. Determination of total lipid classes, and fatty acids in aquatic samples. Pages 4–20 *in* M. T. Arts and B. C. Wainman (editors). Lipids in freshwater ecosystems. Springer, New York.

Parrish, C. C. 2009. Essential fatty acids in aquatic food webs. Pages 309–326 *in* M. T. Arts, M. T. Brett, and M. J. Kainz (editors). Lipids in aquatic ecosystems. Springer, New York.

Pires, M. 2004. Watershed protection for a world city: the case of New York. Land Use Policy 21:161–175.

Potter, B. B., and J. C. Wimsatt. 2005. Determination of total organic carbon and specific UV absorbance at 254 nm in source water and drinking water. Method 415.3. US Environmental Protection Agency, Cincinnati, Ohio.

Pringle, C. M. 1990. Nutrient spatial heterogeneity: effects on community structure, physiognomy, and diversity of stream algae. Ecology 71:905–920.

Pringle, C. M., R. J. Naiman, G. Bretschko, J. R. Karr, M. W. Oswood, J. R. Webster, R. L. Welcomme, and M. J. Winterbourn. 1988. Patch dynamics in lotic systems: the stream as a mosaic. Journal of the North American Benthological Society 7:503–524.

Rasouli, S., J. K. Whalen, and C. A. Madramootoo. 2014. Review: reducing residual soil nitrogen losses from agroecosystems for surface water protection in Quebec and Ontario, Canada: best management practices, policies and perspectives. Canadian Journal of Soil Science 94:109–127.

Smith, L. E., and K. S. Porter. 2010. Management of catchments for the protection of water resources: drawing on the New York City watershed experience. Regional Environmental Change 10:311–326.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd edition. W. H. Freeman and Company, New York, New York.

Solorzano, L., and J. H. Sharp. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnology and Oceanography 25:754–758.

Stanley-Samuelson, D. W. 1994. Prostaglandins and related eicosanoids in insects. Advances in Insect Physiology 24:115–147.

Stevenson, R. J., and L. L. Bahls. 1999. Periphyton protocols. Pages 6.1 to 6.22 *in* M. T. Barbour, J. Gerritsen, and B. D. Snyder (editors). Rapid bioassessment protocols for use in wadeable streams and rivers: periphyton, benthic macroinvertebrates, and fish. 2nd edition. EPA 841-B-99-002. US Environmental Protection Agency, Washington, DC.

Stevenson, R. J., M. L. Bothwell, and R. L. Lowe. 1996. Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California.

Strayer, D. L., R. E. Beighley, L. C. Thompson, S. Brooks, C. Nilsson, G. Pinay, and R. J. Naiman. 2003. Effects of land cover on stream ecosystems: roles of empirical models and scaling issues. Ecosystems 6:407–423.

Thorp, J. H., and M. D. Delong. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos 96:543–550.

Torres-Ruiz, M., J. D. Wehr, and A. A. Perrone. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. Journal of the North American Benthological Society 26:509–522.

Torres-Ruiz, M., J. D. Wehr, and A. A. Perrone. 2010. Are net-spinning caddisflies what they eat? An investigation using controlled diets and fatty acids. Journal of the North American Benthological Society 29:803–813.

USEPA (US Environmental Protection Agency). 1987. Handbook of methods for acid deposition studies. Laboratory analysis of surface water chemistry. EPA 600/4-87/026 Section 21.0. US Environmental Protection Agency, Washington, DC.

USEPA (US Environmental Protection Agency). 2001. Ambient water quality criteria recommendations: information supporting the development of state and tribal nutrient criteria – rivers and streams in nutrient ecoregion VIII. EPA 822-B-01-015. US Environmental Protection Agency, Washington, DC.

WAC (Watershed Agricultural Council). 2011. Strategic plan. New York Council of Nonprofits. Albany, New York. (Available from: https://www.nycwatershed.org/pdfs/Strategic_Plan_2011-2015.pdf)

Wang, L., J. Lyons, and P. Kanehl. 2006a. Habitat and fish responses to multiple agricultural best management practices in a warm water stream. Journal of the American Water Resources Association 42:1047–1062.

Wang, L., D. M. Robertson, and P. J. Garrison. 2006b. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development. Environmental Management 39:194–212.

Watzin, M. C., and A. W. McIntosh. 1999. Aquatic ecosystems in agricultural landscapes: a review of ecological indicators and achievable ecological outcomes. Journal of Soil and Water Conservation 54:636–644.

Whorley, S. B., and J. D. Wehr. 2016. Connecting algal taxonomic information to essential fatty acid content in agricultural streams. Phycologia 55:531–542.

Williams, J. A., A. Sharma, L. J. Morris, and R. T. Holman. 1960. Fatty acid composition of feces and fecaliths. Proceedings of the Society for Experimental Biology and Medicine 105:192–195.

Yates, A. G., and R. C. Bailey. 2010. Covarying patterns of macroinvertebrate and fish assemblages along natural and human activity gradients: implications for bioassessment. Hydrobiologia 637:87–100.

Yates, A. G., R. C. Bailey, and J. A. Schwindt. 2007. Effectiveness of best management practices in improving stream ecosystem quality. Hydrobiologia 583:331–344.

Ziegler, S. E., and D. R. Lyon. 2010. Factors regulating epilithic biofilm carbon cycling and release with nutrient enrichment in headwater streams. Hydrobiologia 657:71–88.