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DOI

[10.1111/j.1529-8817.2008.00503.x](https://doi.org/10.1111/j.1529-8817.2008.00503.x)

Publication date

2008

Published in

Journal of phycology

[Link to publication](#)

Citation for published version (APA):

Veraart, A. J., Romani, A. M., Tornés, E., & Sabater, S. (2008). Algal response to nutrient enrichment in forested oligotrophic stream. *Journal of phycology*, 44(3), 564-572. <https://doi.org/10.1111/j.1529-8817.2008.00503.x>

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ALGAL RESPONSE TO NUTRIENT ENRICHMENT IN FORESTED OLIGOTROPHIC STREAM¹

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Nutrient input in streams alters the density and species composition of attached algal communities in open systems. However, in forested streams, the light reaching the streambed (rather than the local nutrient levels) may limit the growth of these communities. A nutrient-enrichment experiment in a forested oligotrophic stream was performed to test the hypothesis that nutrient addition has only minor effects on the community composition of attached algae and cyanobacteria under light limitation. Moderate nutrient addition consisted of increasing basal phosphorus (P) concentrations 3-fold and basal nitrogen (N) concentrations 2-fold. Two upstream control reaches were compared to a downstream reach before and after nutrient addition. Nutrients were added continuously to the downstream reach for 1 year. Algal biofilms growing on ceramic tiles were sampled and identified for more than a year before nutrient addition to 12 months after. Diatoms were the most abundant taxonomic group in the three stream reaches. Nutrient enrichment caused significant variations in the composition of the diatom community. While some taxa showed significant decreases (e.g., *Achnanthes minutissima*, *Gomphonema angustum*), increases for other taxa (such as *Rhoicosphenia abbreviata* and *Amphora ovalis*) were detected in the enriched reach (for taxonomic authors, see Table 2). Epiphytic and adnate taxa of large size were enhanced, particularly during periods of favorable growth conditions (spring). Nutrients also caused a change in the algal chl *a*, which increased from 0.5–5.8 to 2.1–10.7 $\mu\text{g chl} \cdot \text{cm}^{-2}$. Our results indicate that in oligotrophic forested streams, long-term nutrient addition has significant effects on the algal biomass and community composition, which are detectable despite the low light availability caused by the tree canopy. Low light availability moderates but does not detain the long-term tendency toward a nutrient-tolerant community. Furthermore, the effects of nutrient addition on the algal community occur in spite of seasonal variations in light, water flow, and

water chemical characteristics, which may confound the observations.

Key index words: algal communities; biofilm; chlorophyll; diatoms; light; Mediterranean streams; nutrient enrichment; riparian forest

Abbreviations: BACI, before-after-control-impacted; DCA, detrended correspondence analysis; PCA, principal component analysis; r.i., refractive index; RDA, redundancy analysis

Nutrient availability and light intensity are among the most relevant factors that modulate the dynamics of stream algal communities (Rosemond et al. 1993, Tank and Dodds 2003, Roberts et al. 2004). Anthropogenic factors such as agriculture and urban wastewater enhance nutrient availability (Hilton et al. 2006), causing rich growth of algae and cyanobacteria (Sabater et al. 2000, 2003). These effects are widely reported in open river systems where light does not limit primary productivity or biomass. In these situations, nutrients cause the shift of the community composition toward the dominance of tolerant taxa (Whitton and Kelly 1995, Stevenson and Pan 1999).

Experimental approaches (nutrient addition under controlled conditions in the field) have been performed mostly under nonlimiting light conditions and have produced reports that large increases in biofilm biomass occur immediately after nutrient addition (Peterson et al. 1993, Perrin et al. 1997, Benstead et al. 2005) and affect higher trophic levels (Peterson et al. 1993, Rosemond et al. 1993, Slavik et al. 2004, Benstead et al. 2005). Other ecosystem parameters like secondary production may be affected by nutrient enhancement (Cross et al. 2006). Comparative studies of nutrient addition in open versus shaded systems have shown that algal chl and biovolume accumulation is lower in forested than in clear-cut streams (Lowe et al. 1986, Corkum 1996).

While the effects of nutrient input in open systems are well established, few studies have addressed those in light-limited systems. Given that light is the

¹Received 4 May 2007. Accepted 20 September 2007.

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primary limiting factor for primary production (Corkum 1996, Greenwood and Rosemond 2005), the effects of nutrient enrichment in these ecosystems are expected to be less pronounced. However, it remains to be established whether nutrients simply produce a slower process that leads to a similar effect as under higher light availability. Accordingly, it is unclear whether the response of the algal community in these conditions is homogeneous or species specific, that is to say, whether higher nutrient availability differentially affects some taxa while others remain unaffected. In addition, it remains to be established whether significant descriptors of the algal community, such as density, biovolume, or growth forms, are responsive to nutrient enrichment under light limitation. Finally, it is also of relevance to examine the ways in which natural variation in light, temperature, and water flow in the stream conflicts with the potential effect of nutrients on algal communities. By addressing these questions we seek to contribute to establishing adequate resistance thresholds for nutrient inputs in forested stream ecosystems (Sweeney et al. 2004).

Here we tested the effects of moderate nutrient addition on the epilithic community in an oligotrophic forested stream, characterized by light and nutrient (mostly P) limitation. In this experiment, the stream nutrient regime was manipulated for 1 year, and the effects on the algal community were compared to algal succession previous to the enrichment treatment as well as in that occurring in an untreated upstream reach (Sabater et al. 2005). Because of the Mediterranean character of the stream, the period in which the experiment was performed included marked seasonal changes in hydrology and water temperature and also in light reaching the streambed. The response of the algal community was examined by means of multivariate analyses that consider the overall response of the diatom community and the separate effects of environmental variables.

MATERIALS AND METHODS

Study area. Fuirosos is a third-order stream located in the Montnegre-Corredor Natural Park close to the Mediterranean Sea (northeast Spain, Fig. 1). It has a typical Mediterranean flow regime, with basal flow ranging from 5 to 20 L·s⁻¹ and summer drought (Sabater et al. 2001). Nutrient concentrations (and particularly P) are low in relation to those occurring in disturbed sites of the same area (Romani et al. 2004).

The streambed consists of alternating riffles and pools. Boulders and cobbles are the dominant substrata in riffles, whereas sand accumulates in pools. Branches and leaves are scattered on the streambed and accumulate mainly in riffle areas during low water flow.

The stream banks are steep and covered by dense riparian vegetation that forms a closed canopy. The riparian vegetation consists of alder (*Alnus glutinosa*), hazelnut (*Corylus avellana*), sycamore (*Platanus × hispanica*), and cottonwood (*Populus* sp.; Sabater et al. 2005). Because of the vegetation and the steep banks, light intensity in the river is moderate and heterogeneous throughout the year (Table 1). Daylight average PAR is

usually <20 μE·m⁻²·s⁻¹ for most of the year, except for a short period of time (from March to the end of May) when this parameter can reach values up to 70 μE·m⁻²·s⁻¹ (Acuña et al. 2004).

Experimental design. Two 50 m long stream reaches in the Fuirosos stream were included in the experiment. The two reaches were analogous in terms of slope, type of substrata, and water flow. The upstream untreated control reach (U1) was compared with a downstream reach (E), before and after nutrient enrichment (Fig. 1). U1 was upstream and directly connected to the E reach. Furthermore, the dynamics of the diatom community were also examined in a second reach, U2, located 4,000 m upstream of the other two. Light irradiance, water flow, and water chemistry in U2 differed slightly from the other two reaches; therefore, U2 was not included in the direct analysis of the effects of nutrients on algal dynamics.

Starting in April 2003, the three reaches were monitored monthly for water chemistry and environmental characteristics. From June 14, 2004, onward, nutrients were added continuously to reach E by a flow system consisting of a 200 L reservoir connected to a tap that dripped dissolved nutrients at a constant rate. Nutrient concentration in the tank was adjusted weekly to respond to the variations in basal nutrient concentration in the stream. A small waterfall provided thorough mixing of nutrients and stream water in reach E. N was added as ammonium nitrate, and P as ammonium phosphate. N concentration was increased 2-fold, and P 3-fold with respect to background concentrations. This proportion was chosen to

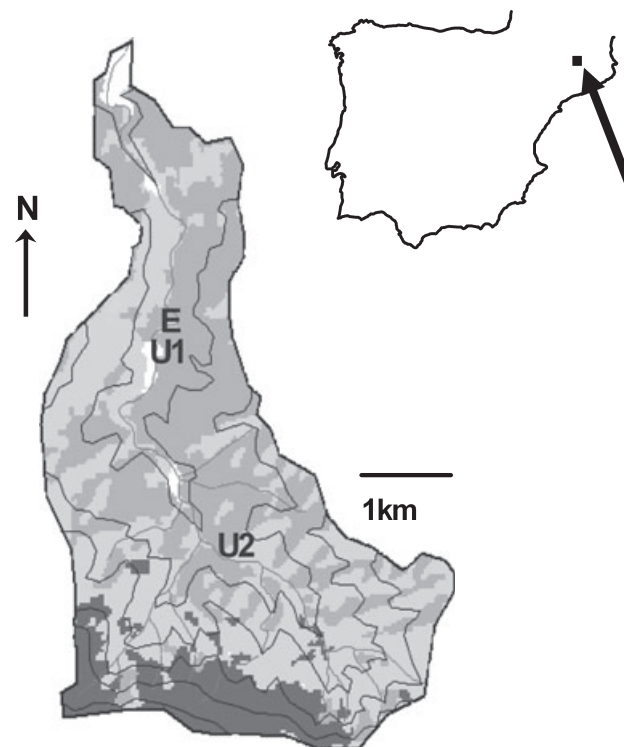


FIG. 1. Map showing the catchment area of the Fuirosos stream and indicating the position of the reaches: E, enriched; U1 and U2, untreated reaches. The inlay shows the position of the stream in Europe. Colors represent vegetation type and soil uses in the watershed: black, deciduous forest; dark gray, coniferous forest; pale gray, evergreen oak forest; white, agricultural fields.

TABLE 1. Environmental variables in the U1 (untreated) and E (enriched) reaches, in the periods before (bef) and after (aft) enrichment of the E reach. The number of samplings included in every period is indicated (*n*).

	U1-bef (<i>n</i> = 4)	U1-aft (<i>n</i> = 6)	E-bef (<i>n</i> = 4)	E-aft (<i>n</i> = 6)
Light ($\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	86.2 ± 80.4	63.8 ± 61.3	106.5 ± 98.0	32.1 ± 23.2
Temperature (°C)	11.2 ± 3.2	11.2 ± 4.5	11.1 ± 3.7	11.9 ± 5.4
Water flow ($\text{L} \cdot \text{s}^{-1}$)	34.3 ± 23.2	19.7 ± 19.1	34.3 ± 23.1	8.9 ± 5.3
Conductivity ($\mu\text{S} \cdot \text{cm}^{-1}$)	179.8 ± 9.1	195.6 ± 44.3	179.5 ± 9.2	222 ± 28.7
P-PO ₄ ($\mu\text{g} \cdot \text{L}^{-1}$)	13.8 ± 16.7	10.4 ± 2.2	16.4 ± 21.7	30.1 ± 13.2
N-NH ₄ ($\mu\text{g} \cdot \text{L}^{-1}$)	26.7 ± 36.3	33.1 ± 40.6	24.6 ± 23.5	185.2 ± 192.6
N-NO ₃ ($\mu\text{g} \cdot \text{L}^{-1}$)	455 ± 223	389.6 ± 185.4	492.0 ± 460.9	580.9 ± 544.9
N/P	77.2	79.9	69.8	56.3

enhance the concentration of dissolved nutrients, in particular P, while striving to maintain the original N:P ratio.

During the study period (April 2003–June 2005), artificial substrata were collected at selected dates for the examination of epilithic communities. The substrata comprised unglazed ceramic tiles (1 × 1 cm) glued to five to six submerged rocks in the riffle sections of each reach. New sets of ceramic tiles were used every 2 months to ensure that algal communities were 2 months old in all sampling periods. The indicative time for algal communities growing on artificial substrata to resemble mature communities colonizing natural substrata is up to 8 weeks (Sabater and Romani 1996). Three colonized tiles were collected at random from distinct rock sets, placed separately in 20 mL glass vials with 10 mL of stream water, and preserved with 4% formaldehyde. Substrata collection was carried out in selected periods, four before the nutrient enrichment period (every 3–4 months) and six afterward. In the post-nutrient-enrichment period, particular attention was given to the periods in which favorable conditions for the algal growth could occur. Therefore, sampling was performed at months 0, 1, 5, 8, 10, 11, and 12, month 0 being the beginning of the nutrient-enrichment experiment (June 2004).

Chl analysis. Three tiles were collected at random from distinct rock sets, kept in a cool box until arrival at the laboratory, and frozen until analysis. Chl *a* was extracted from tiles by sonication (sonication bath operated at 90 s, 40 W power, 40 kHz frequency; Selecta, Barcelona, Spain) of the algal samples. The suspended material was filtered through GF/C glass filters (Sigma-Aldrich, St. Louis, MO, USA) and extracted in 90% acetone. Chl was estimated from spectrophotometric readings following Jeffrey and Humphrey (1975).

Analysis and identification of the algal community. Tiles were sonicated twice for 90 s to separate algae gently (Selecta sonication bath, 40 W, 40 kHz; Sabater et al. 1998). Two separate aliquots for each sample were used for the analysis. In the first, algae (nondiatom) and cyanobacteria were identified to the lowest possible taxonomic level, mainly following Ettl and Gärtner (1988) and Komárek and Anagnostidis (1999, 2005). Algae (nondiatom) and cyanobacteria were not counted, but their relative presence was estimated in a scale ranging from scarce to dominant. The second aliquot was used for diatom identification and counting. Diatom samples were digested in 34% hydrogen peroxide. Permanent slides were made by mounting the clean frustules in Naphrax (r.i. = 1.74). Diatoms were identified to the species level and counted at ×1,000 magnification. Diatom identification was performed mainly following Krammer and Lange-Bertalot (1986–1991) and Lange-Bertalot (2001). Quantification of this algal fraction in relation to the surface area they colonized was achieved by means of subsequent dilutions (using appropriate micropipettes) of the digested fraction. Per each sample, drops of suspended diatom material were prepared, and up to 50 fields in each drop were counted to achieve a minimum of 400 frustules. These numbers were later converted into diatom cells

per cm² and used to estimate the diatom community biovolume (see below). These figures were calculated per sample and replicate. Identification and counting of the algal samples was performed using a Nikon eclipse E600 (Nikon, Tokyo, Japan) light microscope. Nomarski differential interference contrast was used for diatom identification.

The biovolume of the diatom community was estimated after measuring length, width, and height of 10–30 individuals per species and applying a geometric formula to obtain their volume, using the BIOVOL software (Kirschtel 1992, 1996). The total biovolume of the sample was then calculated from the respective composition and densities per surface area of the species in the samples.

Data analysis and statistics. Nondiatom data were estimated qualitatively; therefore, they could not be used in the statistical analyses. Instead, the diatom community was taken to be representative of the quantifiable changes in the whole algal community. Diatom taxa with a relative proportion of >1% and occurring in more than two samples were included in the statistical analyses. Chl data, diatom taxa abundance, and also environmental and nutrient data were log-transformed before further statistical analysis to obtain a normal distribution of the data.

The search for a significant effect of nutrient enrichment on the relative abundance of diatom species was carried out with a multivariate analysis of variance (MANOVA) following a before-after-control-impacted (BACI) design (Underwood 1994) between the diatom communities of U1 and E. These analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

The potential effects of nutrient addition on the diatom community composition were examined by means of multivariate analyses. Data of the three replicates were used in the analyses. Diatom data were analyzed with detrended correspondence analysis (DCA; Hill and Gauch 1980) to determine the length of the gradient for the first two axes. DCA indicated that the gradient length was shorter than 3 SD units (1.7). Therefore, the use of linear ordination techniques was appropriate (ter Braak and Smilauer 2002). Accordingly, principal component analysis (PCA) and redundancy analysis (RDA) were performed using CANOCO version 4.5 (ter Braak and Smilauer 2002). PCA was used to examine the trends of the diatom distribution in the sites. RDA was used to select predictors that best explained the variance of the diatom data.

Our objective was to separate the possible effects of nutrient addition on the diatom communities from those of seasonal variables (light, temperature, water conductivity, water flow) and then to test whether these two groups of variables were redundant to each other or whether they each explained unique aspects of the diatom community structure. Therefore, a series of RDAs and partial RDAs were used to separate and examine the relative importance of two sets of explanatory variables for the species data on the diatom assemblages

(Borcard et al. 1992), one being nutrients, the other being the season-driven variables. These two groups of variables were submitted to the stepwise forward selection procedure in which the statistical significance of each variable was tested by the Monte Carlo permutation test (999 permutations) at a cutoff point of $P=0.05$. Probabilities for multiple comparisons were corrected using Bonferroni correction. Partial RDAs were used to separate and examine the relative importance of each group of explanatory variables for the species data. Variation partitioning was performed in several steps: (i) RDA of the species matrix constrained by the nutrient matrix, (ii) RDA of the species matrix constrained by the environmental (seasonal) variables matrix, (iii) partial RDA of the species matrix constrained by the nutrient matrix and using the environmental variables matrix as covariables, and (iv) partial RDA of the species matrix constrained by the environmental variables matrix and using the nutrient matrix as covariables.

RESULTS

Environmental variables. Water-flow variations followed the pattern of Mediterranean streams. Average water flows (Table 1) ranged between 40 and $70 \text{ L} \cdot \text{s}^{-1}$ from December 2003 to March 2004 and decreased to $2.5\text{--}5 \text{ L} \cdot \text{s}^{-1}$ in summer. The stream dried out for a short period during summer 2003 but not during summer 2004, thereby indicating the characteristic interannual irregularity of the Fuirosos stream (Acuña et al. 2004). No tributaries entered the stream between U1 and E; the water flow of these two reaches was essentially identical ($P=0.329$).

The light regime was determined by the dynamics of the riparian vegetation. As such, maximum values ($130\text{--}200 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) occurred during March–April of the two study years and decreased to $10\text{--}60 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ during the leaf period (May to September) and winter. Occasional higher values were registered in the stream as a result of sunfleck penetration (Sabater et al. 2005). Differences in the light regime were not significant between U1 and E ($P=0.824$). Spring water temperature ranged from 12°C to 14°C , and increased up to 21°C in early summer.

Water conductivity did not show large variations but ranged between 160 and $220 \mu\text{S} \cdot \text{cm}^{-1}$. Temporal variations of conductivity were negatively correlated to water flow ($r=-0.6001$, $P=0.0001$).

Nutrients. Nutrient concentrations in U1 and E fluctuated seasonally, mostly in relation to water flow and organic matter dynamics. Average values of phosphates ranged from 10 to $16 \mu\text{g} \cdot \text{L}^{-1}$, while those of ammonia and nitrates ranged from 25 to $33 \mu\text{g} \cdot \text{L}^{-1}$ and from 390 to $450 \mu\text{g} \cdot \text{L}^{-1}$, respectively. Nitrate was higher during episodes of high flow ($r=0.542$, $P=0.0001$), particularly during autumn–winter. Ammonium concentration increased during November 2004 probably because of the high remineralization rate of organic matter associated with accumulated leaf litter during autumn. During the preenrichment period, nutrient conditions in U1

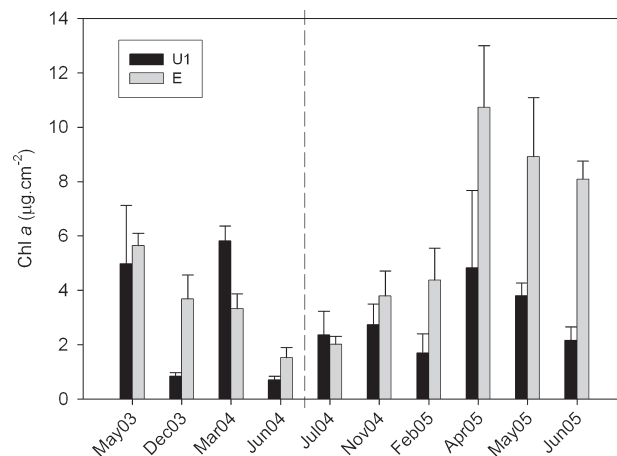


FIG. 2. Chl densities of the algal community in the control reach (U1) and the treated reach (E). Dotted line indicates start of nutrient enrichment.

and E were not significantly different (ammonia, $P=0.137$; nitrate, $P=0.141$; phosphate, $P=0.431$). The enrichment caused the ~ 2 -fold average increase of dissolved N and P (3-fold) in E waters (Table 1). The N:P ratio shifted to from 89 to 56 after the onset of nutrient enrichment.

Chl dynamics. There was a seasonal fluctuation in both U1 and E. Higher values of chl were recorded in spring when they attained $4\text{--}6 \mu\text{g chl} \cdot \text{cm}^{-2}$ in nonenriched conditions. During the preenrichment period, chl concentrations in U1 and E were not significantly different ($P=0.353$). The enrichment caused the chl to increase from values ranging from $0.5\text{--}5.8$ to $2.1\text{--}10.7 \mu\text{g chl} \cdot \text{cm}^{-2}$ (correlated to P concentration, $r=0.412$, $P=0.001$; Fig. 2). The chl values in the postenrichment period were significantly different between the E and U1 reaches ($P=0.013$; Fig. 2).

Cyanobacterial and algal community composition. The nondiatom assemblage was dominated by Chroococcales (mainly *Pleurocapsa* spp., *Chroococcus* spp., and *Gloeocapsa* sp.), filamentous cyanobacteria (mostly *Homeothrix varians* Geitler), and Chlorococcales (mostly *Chlorococcus* sp. and *Gongrosira* sp.). Differences between the occurrence of nondiatom taxa in the three reaches were not remarkable. However, the filamentous green alga *Cladophora glomerata* (L.) Kütz. was occasionally found in the E reach after nutrient addition but was absent in the others.

Diatom density and biovolume. The diatom community showed the lowest densities in winter ($0.37\text{--}1.37 \times 10^4 \text{ cells} \cdot \text{cm}^{-2}$), while maxima occurred in spring ($2.4\text{--}9.7 \times 10^6 \text{ cells} \cdot \text{cm}^{-2}$; Fig. 3A). There was a slight decrease in cell density in E with respect to U1, though differences were not statistically significant ($P=0.720$).

After nutrient addition (and in particular during spring and early summer), the diatom biovolume in E was almost three times higher than in U1. Average biovolumes were 6.50 ± 11.16 and $1.44 \pm 3.08 \times$

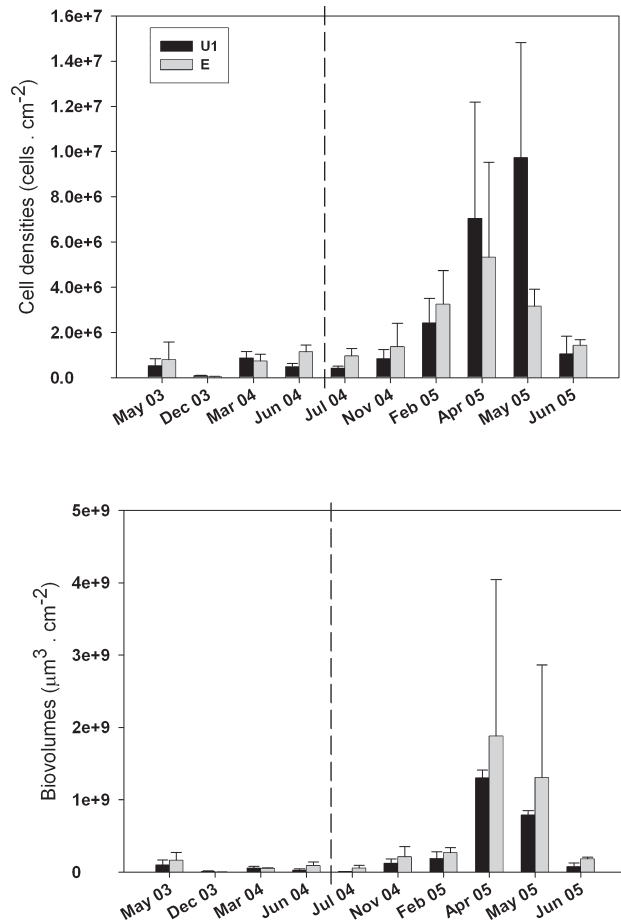


FIG. 3. Cell densities (upper) and biovolumes (lower) of the diatom community in the control reach (U1) and the treated reach (E). Dotted line indicates start of nutrient enrichment.

$10^8 \mu\text{m}^3 \cdot \text{cm}^{-2}$ in the former and latter, respectively (Fig. 3B). However, this variation was not statistically significant ($P = 0.318$).

Diatom community composition. Diatoms were the dominant taxonomic group in the Fuirosos stream. Ninety-seven diatom taxa were identified in the sample set. The average percent contribution of the most frequent taxa (those $>1\%$ in at least two samples) is shown in Table 2 in the periods before and after nutrient input.

While most taxa occurred in low numbers, a few (10 taxa) comprised 93% of the total cell densities (Table 2). The community composition in U1 and U2 and E showed the same dominant taxa, and these reaches were dominated in all cases by large numbers of the adnate taxa *Ac. minutissima* and *Cocconeis placentula*. The differences between U1 and U2 were related mostly to the relative proportion of *Ac. minutissima*, which was greater in the reach further upstream.

The relative abundance of five taxa differed significantly between E and U1, as shown by means of a BACI MANOVA. Percent abundances of *Ac. minutissima*, *Ac. lanceolata* var. *frequentissima*, and

G. angustum decreased ($P = 0.046$, 0.003 , and 0.04 , respectively) in E with respect to U1. However, those of *R. abbreviata* and *Gomphonema minutum* ($P = 0.008$ and 0.013 , respectively) increased (Fig. 4, A–D).

The distinct sensitivity of the species to nutrient addition were also reflected by the intensity of the changes recorded in their abundances. *Ac. minutissima* showed an average decline of 62%, while *R. abbreviata* increased in a similar proportion. In contrast, several other taxa showed only minor differences between the E and U reaches, among these *G. minutum* and *G. angustum*.

A PCA was performed with samples from U1 and E, considering the percent abundance of the 27 taxa that accounted for a minimum of 1% abundance in at least two samples. The first axis of the PCA (15% of the variance) showed mostly the separation between the diatom taxa characteristic of E during winter and spring (*Co. placentula*, *R. abbreviata*, *G. minutum*), from U1 but also E before nutrient addition (*Navicula cryptocephala*, *G. angustum*). The PCA results therefore showed that nutrient addition produced a major change in the diatom communities of E.

RDAs were therefore run on the same 27 diatom taxa from 60 samples, three nutrient forms (N-NH₄, N-NO₃, and P-PO₄), and four other environmental variables (light, temperature, water flow, and conductivity; Table 3). The three nutrient variables and also all the environmental variables (except conductivity) were retained (Fig. 5). The first RDA axis (RDA1; 16.3% of the variance) showed a strong correlation with nitrate and flow (which indicates the seasonal pattern of this nutrient) as opposed to ammonia concentration (related to the nutrient addition). *Co. placentula*, *R. abbreviata*, and *Fragilaria ulna* were the taxa most related to ammonia abundance and conditions of low flow. These were characteristic taxa not only in samples from E after the enrichment, but also in samples from U1 during February and November. *Ac. minutissima* and *Gomphonema pumilum* were the taxa less favored by ammonia abundance but were, in contrast, closely correlated to the water flow and nitrate abundance. This observation was common in samples from U1 (mostly during winter and early spring) and from analogous conditions in E before nutrient enrichment.

The second RDA axis (RDA2) explained a low proportion of the variance (4.8%) and was mostly negatively related to light, temperature, and phosphate concentration. These relationships indicate that favorable growth conditions (high light and temperature) are accompanied by the abundance of phosphate. In the negative part of the axis were samples corresponding to spring and winter, mostly in E. The subdominant taxa *Am. ovalis* and *Reimeria sinuata* were the most enhanced by these conditions.

The relevance of seasonality and nutrients on the distribution of the diatom assemblages in the sites

TABLE 2. Percent abundances of the most frequent taxa in three reaches of the Fuirosos stream. U1 and U2 untreated; E, enriched both in the periods before (bef) and after (aft) the enrichment of the E reach. Acronyms identifying the taxa are indicated.

		U1-bef	U1-aft	U2-bef	U2-aft	E-bef	E-aft
<i>Achnanthes biasolettiana</i> Grunow	ABIA	1.1	1.2	0.4	0.1	5.4	0.7
<i>Achnanthes lanceolata</i> (Bréb.) Grunow	ALAN	7.2	1.3	5.7	7.1	7.3	2.2
<i>Achnanthes minutissima</i> Kütz.	AMIN	56.2	55.0	81.2	71.4	52.2	34.5
<i>Achnanthes lanceolata</i> v. <i>frequentissima</i> Lange-Bert.	ALFR	0.0	0.1	0.1	0.9	1.0	0.0
<i>Amphora inariensis</i> Krammer	AINA	1.9	2.0	0.3	0.1	1.0	1.4
<i>Amphora ovalis</i> (Kütz.) Kütz.	AOVA	0.0	0.0	0.0	0.0	0.0	1.3
<i>Amphora pediculus</i> (Kütz.) Grunow	APED	0.9	2.2	0.1	0.6	3.3	1.3
<i>Cocconeis pediculus</i> Ehrenb.	CPED	0.1	0.2	0.0	0.1	0.2	1.7
<i>Cocconeis placentula</i> Ehrenb.	CPLA	13.7	20.3	1.8	8.4	12.3	32.4
<i>Cymbella silesiaca</i> Bleisch in Rabenh.	CSLE	0.0	0.2	0.0	0.1	0.0	0.1
<i>Diploneis oblongella</i> (Nägeli) Cleve-Euler	DOBL	0.6	0.0	0.9	0.2	0.0	0.0
<i>Fragilaria capucina</i> var. <i>gracilis</i> (Øestrup) Hustedt	FCCP	0.3	1.0	0.1	0.0	0.1	3.5
<i>Fragilaria capucina</i> var. <i>capitellata</i> (Grunow) Lange-Bert.	FCCP	0.0	0.2	0.0	0.1	0.4	0.6
<i>Fragilaria ulna</i> (Nitzsch) Lange-Bert.	FULN	0.2	1.1	0.1	0.1	0.0	1.4
<i>Gomphonema angustatum</i> (Kütz.) Rabenh.	GADI	1.6	0.6	1.6	1.8	1.0	0.8
<i>Gomphonema angustum</i> C. Agardh	GANT	0.2	0.1	0.6	1.2	1.4	0.1
<i>Gomphonema minutum</i> (C. Agardh) C. Agardh	GMIN	0.2	0.0	0.3	0.1	0.3	0.5
<i>Gomphonema pumilum</i> (Grunow) E. Reichardt et Lange-Bert.	GPUM	1.7	0.0	2.0	1.4	1.1	0.0
<i>Meridion circulare</i> (Grev.) C. Agardh	MCIR	0.0	0.1	1.5	1.7	0.4	0.2
<i>Navicula antonii</i> Lange-Bert.	NANT	0.8	0.1	0.1	0.2	0.8	0.2
<i>Navicula accomoda</i> Hust.	NARB	0.0	0.0	0.0	0.0	0.0	0.0
<i>Navicula capitatoradiata</i> H. Germ.	NCPR	0.3	0.0	0.1	0.1	0.0	0.3
<i>Navicula cryptocephala</i> Kütz.	NCRY	0.5	0.1	0.1	0.3	0.5	0.2
<i>Nitzschia linearis</i> (C. Agardh) W. Sm.	NLIN	0.2	0.0	0.0	0.2	0.0	0.1
<i>Nitzschia palea</i> (Kütz.) W. Sm.	NPAL	0.0	0.0	0.3	0.2	0.5	0.2
<i>Reimeria sinuata</i> (W. Greg.) Kociolek et Stoermer	RSIN	3.4	2.0	0.6	1.2	4.9	2.8
<i>Rhoicosphenia abbreviata</i> (C. Agardh) Lange-Bert.	RABB	8.1	5.0	0.2	0.4	3.6	9.9

was analyzed by means of partial RDAs. A first RDA examined the effect of nutrients on taxonomic structure. This RDA selected the three nutrient forms as significant variables, which accounted for 7.8% of the total variance. A second RDA considered the effect of the environmental variables on the diatom communities. Temperature, light, and flow were significant and explained 8.6%. The covariance of the two groups of factors (i.e., the variance conjointly explained by nutrients and the environmental variables) accounted for 10.6%. The unexplained variance accounted for 73%.

DISCUSSION

Moderate nutrient addition to a forested stream (in a low light system) caused significant long-term changes in the biomass (chl *a*) and composition of the algal community. Chl *a* enhancement as a result of nutrient enrichment under low light conditions has been also detected in other forested streams (Greenwood and Rosemond 2005), although a longer time was required to achieve greater biomass than in open stream systems (Guasch and Sabater 1995). This study highlighted that nutrient effects were not immediate and did not homogeneously affect the algal taxa. While some taxa were enhanced, others were affected negatively. Moreover, nutrient addition did not produce a significant shift in cell density and biovolume, implying that the effects on the algal community (including the

nondiatoms) were moderate. Furthermore, the changes observed in the diatom community occurred in spite of the superimposed seasonal variability of the environmental variables, which includes relevant alterations in light, temperature, and water flow both annually and interannually (Acuña et al. 2005).

The effects on particular taxa were confirmed by means of several multivariate analyses. The ANOVA performed with the BACI design on the most frequent diatom taxa showed that a small number, including both dominant and subdominant, were affected by nutrient addition. These results were largely coincident with those of the RDA, which determined the proportion of variance of each taxon that was predicted by the nutrients or by other environmental factors (light, temperature, water flow, water conductivity). Other factors that were not considered (e.g., grazing; Sabater et al. 2005) could obviously account for a relevant proportion of the unexplained variance. The variance partition is a useful tool to determine taxa predictors, particularly in data sets collected in a narrow range of environmental factors (Leira and Sabater 2005, Passy 2006). In our study, >50% of the variance of nine taxa was explained by nutrients. Among these, some were dominant, their occurrence being limited (*Ac. minutissima*) or favored (*R. abbreviata*) by the nutrients; others were subdominant (*G. minutum*, *G. pumilum*) or showed small pulses in response to nutrient enrichment (*Navicula accomoda*, *Am. ovalis*).

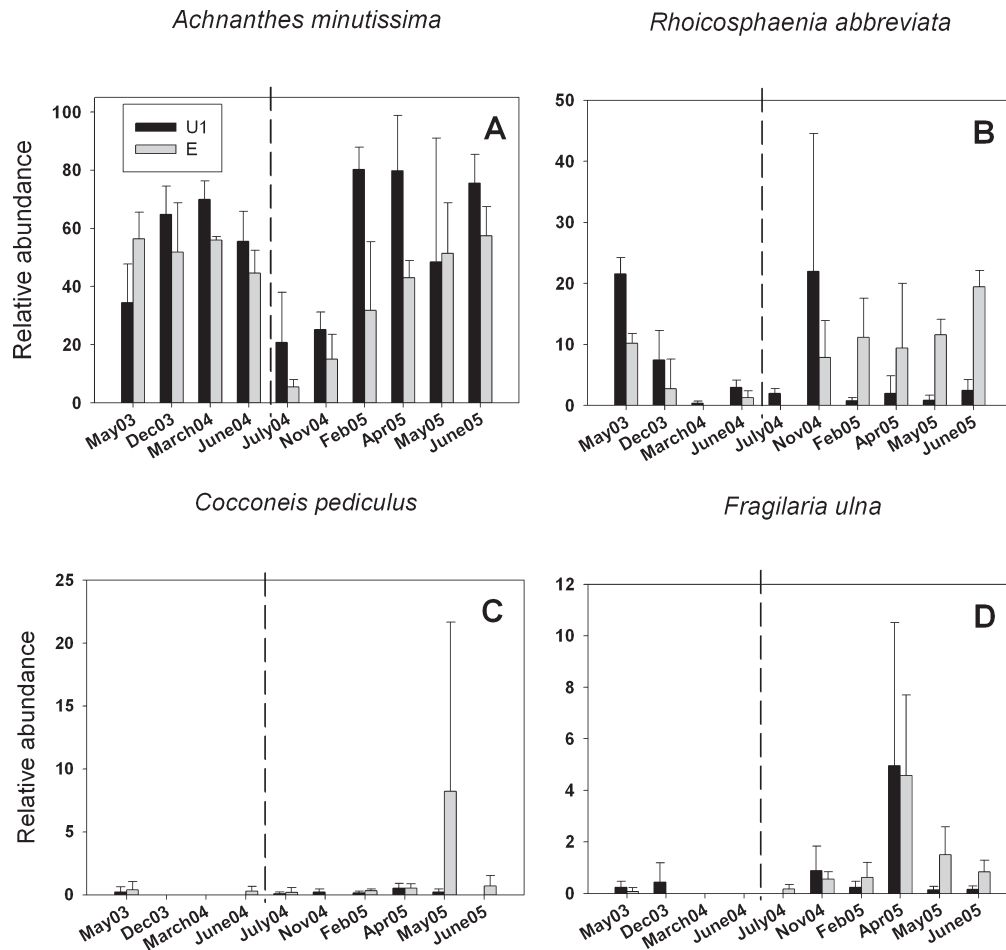


FIG. 4. Temporal changes in percent abundances of selected diatom taxa (*Achnanthes minutissima*, *Cocconeis pediculus*, *Rhoicosphaenia abbreviata*, and *Fragilaria ulna*), for the control (U1, black bar) and enriched reach (E, gray bar). Note that the abundance scales are not the same in the different taxa. Error bars show standard deviations. Dotted line indicates start of nutrient enrichment.

The changes observed in the diatom community in E cannot be related to the success of a particular algal growth form. Occurrence of growth forms has been associated with grazing pressure, the adnate forms showing more resistance to grazers than loosely attached taxa (Hill and Knight 1987). Analogously, stalked or filamentous taxa may be more sensitive to nutrient addition because of their prevalence in the spatial arrangement of the algal community (de Nicola et al. 2006). In our experiment, both stalked *Gomphonema* and adnate *Achnanthes* showed high sensitivity to nutrient enrichment. However, the enriched reach contained a higher proportion of epiphytic species, possibly because of the increased abundance of the filamentous green alga *Cl. glomerata* and its potential for hosting epiphytes. *Cladophora* enhances the three-dimensional structure of the biofilm, thereby providing a greater surface for colonization (Dodds 1991, Mpawenayo and Mathooko 2005).

The differential response of species to nutrient enrichment is difficult to explain. In our experiment, some species, such as *Ac. minutissima* and

Co. pediculus, showed a strong response to this treatment (negative and positive, respectively), whereas several others showed only minor responses, or responses that were detectable only after a longer period of time. *Ac. minutissima* can be considered a fast colonizer, and its rapid response (and high sensitivity) to nutrient enrichment is possibly related to its colonization capacity (Stevenson et al. 1991). Success of *Co. pediculus* could be a subsequent result of the moderate increase of *Cl. glomerata*. Response to nutrients might also be determined by the particular physiological preferences of taxa, which may be associated with the surface area to volume ratio (Steinman et al. 1997). Greater nutrient uptake is reflected by higher growth rates (Tilman and Kilham 1976). However, on the basis of our data, there is little evidence that nutrient enhancement produced an increase in the growth rate of the diatom community. Nevertheless, there was a greater occurrence of larger taxa that produced an increase in the community biovolume. This increase occurred mostly during short pulses of light availability, registered in late fall-winter and in early spring. In these

TABLE 3. Results of the partial redundancy analyses (RDAs), with the percentage of the total variance explained by environmental and nutrient factors with respect to the total and constrained variance.

Taxon acronyms ^a	Fraction total variance		Fraction explained variance	
	Nutrients	Environmental	Nutrients	Environmental
ABIA	7.0	12.9	35.1	64.9
ALAN	15.9	32.3	33.0	67.0
AMIN	16.9	6.8	71.2	28.8
ALFR	2.8	7.4	27.2	72.8
AINA	3.3	8.8	27.1	72.9
AOVA	45.8	3.6	92.8	7.2
APED	7.7	9.9	43.9	56.1
CPED	17.4	5.3	76.7	23.3
CPLA	2.2	7.3	23.4	76.6
CSLE	4.2	9.8	29.7	70.3
DOBL	1.2	3.6	25.6	74.4
FCCP	0.7	4.2	15.0	85.0
FCCP	5.5	3.5	60.9	39.1
FULN	17.3	5.7	75.4	24.6
GADI	14.2	6.6	68.1	31.9
GANT	9.8	28.4	25.7	74.3
GMIN	15.1	9.9	60.5	39.5
GPUM	6.1	8.8	41.1	58.9
MCIR	15.2	29.9	33.7	66.3
NANT	2.2	12.6	15.0	85.0
NARB	1.8	1.1	61.5	38.5
NCPR	2.5	3.4	42.5	57.5
NCRY	9.0	17.9	33.5	66.5
NLIN	4.9	6.5	43.2	56.8
NPAL	3.0	3.6	45.0	55.0
RSIN	2.0	15.6	11.5	88.5
RABB	4.5	4.4	51.0	49.0

^aFor taxonomic names in full, see Table 2.

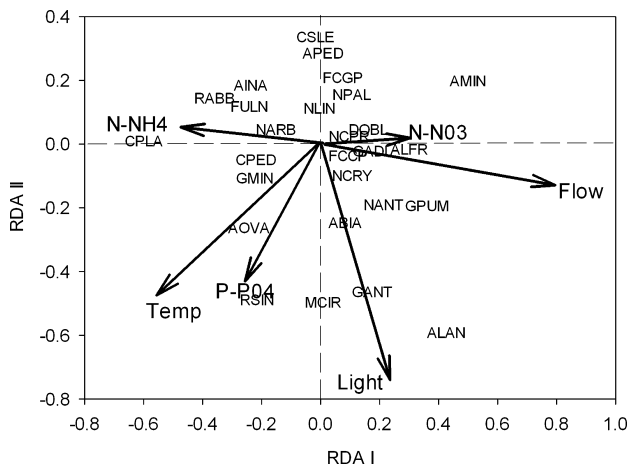


FIG. 5. RDA analysis results performed with the percent abundances of the 27 most frequent species. See Table 2 for explanation of species acronyms.

periods, higher light and nutrient availability (in the E reach) affected the diatom community by favoring communities of larger size taxa, such as *F. ulna*.

The combination of factors that predicted the higher occurrence of particular taxa was expressed in our study by the large covariance between nutrients and other environmental factors (39.3% of the

explained variance). These factors were light, water flow, and temperature. Algae inhabiting forested streams have the capacity to acclimate to low light intensity (Roberts et al. 2004). These light conditions affect their photosynthetic efficiency but do not impair growth rates, in particular, in the case of thin diatom-dominated communities (Rier et al. 2006). In Mediterranean forested streams, the pulses of light availability are highly significant for primary producers, mostly because water temperature does not limit algal growth (Guasch and Sabater 1995). Nutrients and light availability may have exerted combined effects in the enriched reach during March–April 2005. In spring 2005, PAR on the streambed was the highest, thereby explaining the favorable conditions for algal growth, mostly in the enriched section. Nutrient dynamics cannot be separated from changes in water flow, in particular because of large storm events (Butturini et al. 2005) and because of resumed flow after extended drought periods (Romaní et al. 2006). During our study period, the high remineralization of organic matter accumulated in the streambed caused high natural ammonia concentrations during November 2004. The nutrient enrichment in addition to this natural ammonia pulse produced the isolated occurrence of nutrient-tolerant species such as *Navicula accomoda*. Nevertheless, these outgrowths occurred in low numbers.

In conclusion, the effects of nutrient addition in the oligotrophic forested stream were noticeable mostly in the biomass and algal community composition, even in the low light environment caused by the tree canopy and shading from steep stream banks. Epiphytic diatom species were mostly favored by the increased nutrient levels. Changes observed in this ecosystem were not pronounced and required a long period of time to manifest, thereby increasing the probability of confounding effects of seasonal events.

We thank Joan Artigas and Irene Ylla for help with fieldwork, Gemma Urrea for her assistance with diatom identification and diatom slide preparation, and Wim Admiraal for helpful discussion and critical reading of the manuscript. Annelies Veraart received grants from the EU Socrates/Erasmus programme and the Dr. C. Buisman Foundation. This research was funded by the project CGL2005-06739-CO2/BOS and by the FBBVA project GLOBRIO.

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