

Effects of flow events and nutrient addition on stream periphyton and macroinvertebrates: an experimental study using flumes

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Abstract – We used flume experiments to study the effects of a temporary increase in stream flow on macroinvertebrates, leaf litter breakdown and soft-bodied benthic algae; both as a single stressor and eventually in combination with an increase in nutrient supply. In order to understand how well the flumes reflected the nearby stream ecosystem, we compared species composition of macroinvertebrates and benthic algae between the flumes and the nearby stream from which the flumes were supplied with water. As single stressors, nutrient addition and an increased flow velocity from 1.3 to 2.8 cm s⁻¹ lead to an increase in the biomass of benthic algae, likely reflecting an improved transfer of nutrients into algal patches. However, the combined effect of flow and nutrient addition was smaller than the sum of both individual effects, likely because an increased biomass also was more susceptible to scouring. We found differences in macroinvertebrate and benthic algal taxon identity and abundance between stream and flumes. Since biodiversity is assumed to stabilize ecological functioning in response to disturbances and variation, we conclude that care should be taken in applying results from small scale experiments to stream ecosystems.

Keywords: river flow / eutrophication / periphyton / macroinvertebrates / flume experiment

Résumé – Effets des épisodes d'écoulement et de l'adjonction de nutriments sur le périphyton et les macroinvertébrés des cours d'eau : une étude expérimentale utilisant des canaux. Nous avons utilisé des expériences en canaux pour étudier les effets d'une augmentation temporaire du débit sur les macroinvertébrés, la dégradation de la litière foliaire et les algues benthiques à corps mou, à la fois comme stressor unique et éventuellement en combinaison avec une augmentation de l'apport en éléments nutritifs. Afin de comprendre dans quelle mesure les canaux reflétaient bien l'écosystème du cours d'eau voisin, nous avons comparé la composition spécifique des macroinvertébrés et des algues benthiques entre les canaux et le cours d'eau voisin qui alimentait les canaux en eau. En tant que stressors uniques, l'ajout de nutriments et une vitesse d'écoulement accrue de 1,3 à 2,8 cm s⁻¹ entraînent une augmentation de la biomasse des algues benthiques, ce qui reflète probablement un meilleur transfert des nutriments dans le périphyton algal. Toutefois, l'effet combiné du débit et de l'adjonction de nutriments était plus faible que la somme des deux effets individuels, probablement parce qu'une biomasse accrue était aussi plus susceptible de se décoller. Nous avons trouvé des différences dans l'identité et l'abondance des taxons de macroinvertébrés et des taxons d'algues benthiques entre le cours d'eau et les canaux. Puisque la biodiversité est supposée stabiliser le fonctionnement écologique en réponse aux perturbations et aux variations, nous concluons qu'il faut faire preuve de prudence dans l'application aux écosystèmes des cours d'eau des résultats d'expériences à petite échelle.

Mots-clés : débit fluvial / eutrophisation / périphyton / macroinvertébrés / canal expérimental

1 Introduction

Stream ecosystems may respond to stressors through changes in their ecological structure and function (Gucker

et al., 2006; Hogsden and Harding, 2012). Traditionally, scientific studies have focused on the impact of one primary stressor on aquatic ecosystems (e.g. Biggs, 2000; Dangles and Guérol, 2000). In recent years, however, there have been attempts to understand how multiple stressors may simultaneously affect ecosystems, since this reflects more realistic conditions (e.g. Townsend *et al.*, 2008; Ormerod *et al.*, 2010).

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In Europe, pollution from agriculture and hydraulic engineering, *i.e.* the modification of the natural flow regime, are regarded as the main factors inhibiting the achievement of good ecological status of river basins (Menendez *et al.*, 2005). The excessive use of fertilizer in agriculture may cause stream eutrophication, *i.e.* the accelerated growth of plants and algae caused by an increased nutrient loading (Schneider *et al.*, 2016). The effects of eutrophication on the structure and function of stream ecosystems are relatively well understood, and commonly include an increase in periphyton biomass (Biggs, 2000), changes in species composition of macrophytes and benthic algae (Kelly and Whitton, 1998; Schneider and Melzer, 2003), or a decline in macrophyte species richness (Sand-Jensen *et al.*, 2000). The change in the rate of leaf litter breakdown with nutrient enrichment is not as clear; as it has been shown to both increase (*e.g.* Greenwood *et al.*, 2007) and decrease (*e.g.* Lecerf *et al.*, 2006). Macroinvertebrate species richness may be lowered if certain species tend to predominate in response to nutrient loading (Lecerf *et al.*, 2006; Stendera *et al.*, 2012), or increase, if higher nutrient levels allow for the establishment of a more complex ecosystem (*e.g.* the intermediate disturbance hypothesis as proposed by Connell (1978)).

The flow regime is often regarded to be the key abiotic variable in river ecosystems (Poff *et al.*, 1997; Bunn and Arthington, 2002). River flow is, however, notoriously variable. This variability may be related to natural factors such as climate, geology and topography. Long-term climate change is – in the Northern Hemisphere – expected to lead to an increase in the frequency and intensity of heavy precipitation events, thus increasing the magnitude of high flows (Fowler and Kilsby, 2007; IPCC, 2013). Natural fluctuations in river flow are fundamentally important for the ecological integrity of lotic ecosystems (Poff *et al.*, 1997; Naiman *et al.*, 2008). The flow regime of many rivers has, however, been modified, *e.g.* by dampening or eliminating natural floods and droughts in order to meet human needs such as transport, water supply, flood control or hydropower (Dynesius and Nilsson, 1994; Gleick, 2003). Such an anthropogenic modification often leads to a more uniform flow regime, including reduced peak discharges (Graf, 2006). The consequences of natural variation and anthropogenic modifications in flow to riverine biota have been relatively well studied (Rolls *et al.*, 2012; Schneider and Petrin, 2017), and both flow maxima and minima are important. For example, spates can decrease the abundance and diversity of macroinvertebrates (Scrimgeour *et al.*, 1988), but macroinvertebrate assemblages and trait composition are also affected by droughts (Monk *et al.*, 2008; Bonada *et al.*, 2007).

While the separate effects of nutrients and a change in stream flow are relatively well understood, the combined effects have received less attention. Matthaei *et al.* (2010) used flume experiments to study the interacting effects of water abstraction (*i.e.* reduced flow) and nutrient addition. They commonly observed single stressor effects of nutrients and reduced flow on macroinvertebrates, benthic algal biomass and leaf litter degradation. Significant interactions, however, were comparatively rare, and were only observed for macroinvertebrates, not for benthic algae or leaf litter degradation. Elbrecht *et al.* (2016) did a similar study in Europe, and the results supported Matthaei *et al.* (2010). Also Lange *et al.*

(2014) studied the effect of farming intensity and water abstraction on macroinvertebrate communities in 43 stream sites. However, farming intensity not only affected nutrient concentrations, but also fine sediment levels on the stream bed, as well as temperature and light conditions. At the same time, water abstraction not only affected water flow, but also enhanced the accumulation of fine sediments and changed temperature. These covariations made it difficult to infer possibly causal relationships between macroinvertebrates and the combined effects of changes in nutrient levels and flow variation.

We used flume experiments in order to study the effects of a temporary increase in river flow, *e.g.* reflecting a heavy precipitation event, both as a single stressor and in combination with an increase in nutrient supply. This is important, because forecasted climate change may not only lead to an increase in the frequency of heavy precipitation events (Fowler and Kilsby, 2007; IPCC, 2013), but also increase nutrient exports from agricultural land (Ulén *et al.*, 2007). We focused on analysing the responses of macroinvertebrates, benthic algae and leaf litter decomposition, because they are generally regarded as key components of stream ecosystems (Matthaei *et al.*, 2010). We also compared species composition of macroinvertebrates and soft-bodied benthic algae between the flumes and the nearby stream from which the flumes were supplied with water, in order to understand how well the flumes represented the corresponding stream ecosystem. That comparison was important because small scale experiments have long been criticised for giving highly replicable, but spurious, answers (Schindler, 1998).

We hypothesized that (1) the flumes would contain fewer invertebrate and periphyton taxa than the nearby stream, because the flumes represent artificial and uniform habitats; (2) the flow event would decrease the biomass and diversity of benthic algae and invertebrates due to scouring, (3) nutrients would increase the biomass of benthic algae and decrease the rate of leaf litter breakdown (Robinson and Gessner, 2000), and (4) a flow event in the high nutrient treatment would reduce benthic algae more than in the low nutrient treatment, because nutrient enriched habitats should have an increased biomass of algae which generally are assumed to be more susceptible to flow (Biggs *et al.*, 1998a).

2 Material and methods

2.1 Study site

The flumes were located at Sagelva (63.135° N, 9.853° E), a stream near Trondheim, Norway, downstream from a small waterfall on a small stream island. The study site was located approximately 1 km downstream from a lake outlet. The catchment vegetation in the vicinity of the study site mainly comprised coniferous forest trees, but broad-leaf trees, *Betula* sp., *Salix* sp., and *Alnus* sp., also grew in the riparian zone. Human activities in the catchment mainly entailed forestry and agriculture. During the experiments, the water in Sagelva was circumneutral (pH = 7.7–7.8). Total organic carbon ranged from 4.0 to 4.6 mg C l⁻¹, calcium from 13.4 to 13.9 mg l⁻¹, total phosphorus from 2 to 7 µg P l⁻¹, total nitrogen from 235 to 370 µg N l⁻¹, and nitrate from 102 to 138 µg N l⁻¹, respectively (own unpublished data obtained from four

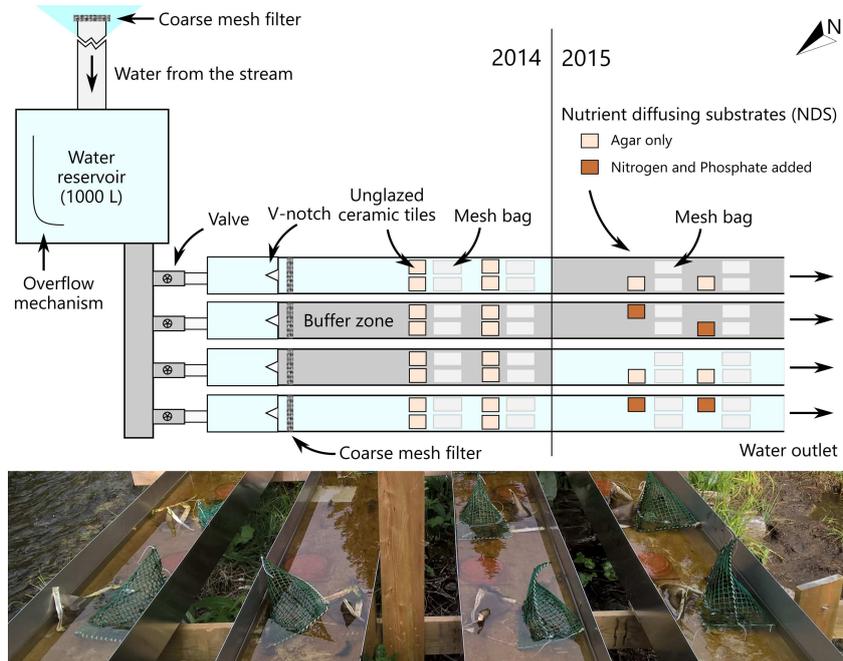


Fig. 1. Flume design and experimental setup in 2014 and 2015. In 2014, we used unglazed ceramic tiles and mesh bags. In 2015, we replaced the tiles with NDS. In each flume, there were two stations, one sampled prior to and one sampled after the flow event. Treatment, tiles and mesh bags were randomly distributed in both years and both experimental rounds.

samples taken during the study period; all measurements were performed in an accredited laboratory according to Norwegian standard procedures).

2.2 Experimental flumes

We constructed four metal flumes, each 4 m long and 32 cm wide, that were equipped with water delivery containers with large diameter taps allowing for separate manipulation of water flow in each flume (Fig. 1). Water was supplied to all flumes from a large delivery tank with an overflow mechanism, which was connected to a pool in Sagelva, guaranteeing a constant supply of water throughout the experimental period independent of the actual water level in the stream. To measure and help adjust discharge in each flume, we installed a V-notch and a tap in each flume, such that the water level behind the V-notch corresponded to a defined discharge. Filtering devices were attached both at the tip of the supply pipe to the main delivery tank and below the V-notches to ensure that no coarse material could enter the flumes that might have clogged the experimental setup. The bottoms of the flumes included no mineral or organic substrate except for the experimental components. The flumes were exposed to sunlight and hence experienced the same light regime along their entire lengths.

The experiments were repeated twice in 2014, when we studied the effects of flow as a single stressor, and in 2015, when we studied the combined effects of flow and nutrient enrichment. The first experimental round was performed in July/August with the second round in August/September. During each experimental round, the flumes were operated for approximately two weeks following a pre-exposure period to allow for the development of a periphyton layer in the flumes (Tab. 1).

The base flow was in all experiments in both years sufficient to keep all experimental components in the flumes submerged. In order to study the effect of flow as a single stressor in 2014, we set two hydrological regimes. Base flow was set to 0.22 l s^{-1} (corresponding to a flow velocity of 2.9 cm s^{-1}) in all flumes and maintained throughout the experiment in two randomly selected flumes serving as controls. In the remaining two flumes, discharge was increased to 0.77 l s^{-1} (corresponding to a flow velocity of 6.9 cm s^{-1}) for the duration of 3 days between the first and the second sampling, simulating a flow event that may for instance occur after rainfall. Thus, discharge during the event flow was ≈ 3.6 -fold larger than during base flow. The water depth was 2.3 cm at base flow and 3.5 cm during the event flow.

In order to study the combined effects of nutrients and flow in 2015, we manipulated nutrient levels in addition to the discharge levels. To allow for complete submersion of nutrient diffusing substrates (NDS), the water depth in the flume had to be increased by installing a threshold at the end of each flume. This increased the water depth at base flow to 3.5 cm. Base flow was set to 0.07 l s^{-1} (corresponding to a flow velocity of 1.3 cm s^{-1}). During the flow events water flow was 0.93 l s^{-1} (corresponding to a flow velocity of 2.8 cm s^{-1}). Water depth during the event flow was 4.8 cm.

We installed drift nets with a mesh size of $250 \mu\text{m}$ at the ends of the flumes to examine whether benthic macroinvertebrates may have drifted out of the flumes. Sampling was timed to begin with the onset of the flow event and lasted for one hour. All macroinvertebrates that were collected in the drift nets were preserved in 70% ethanol for later identification in the laboratory.

Last, we installed temperature loggers (HOBO Pendant Temperature/Light Data Logger, Onset Computer Corp.) in the

Table 1. Timing of sampling and duration of pre-exposure of mesh bags and tiles (2014) or nutrient diffusing substrates (2015). The start of the flow event is set as day 0. Sampling 1 was performed before the onset of the flow event. MB = mesh bags, NDS = nutrient diffusing substrates.

Year	Round	Exposure of tiles/NDS in flumes	MB in stream	MB in flumes	Sampling 1	Flow event	Sampling 2
2014	1& 2	-13	-6 to -3	-3	0	0-3	7
2015	1*	-3	-6 to -3	-3	0	0-3	8
2015	2**	-7	-11 to -7	-7	0	0-3	7

* Pre exposure time had to be reduced compared to 2014 due to a partial collapse in the supporting structure of the flumes.

** Due to clogging of one of the valves, the experiment was restarted after one week, reducing the pre-exposure time of the NDS and extending the time the mesh bags were in the flumes prior to the flood.

stream and in one of the flumes during the experiments performed in 2015, to test if water temperature would differ between the stream and the flumes and if this could explain potential differences in macroinvertebrate and algal assemblages between the stream and the flumes.

2.3 Response parameters

We used mesh bags filled with 1.5 g dried grey alder (*Alnus incana*) leaves to measure leaf litter decomposition (Benfield, 1996; Gessner and Chauvet, 2002). We used two mesh sizes, 0.5 µm (=excluding benthic macroinvertebrates) and 1.2 cm (=allowing macroinvertebrates access to the leaf litter). After sampling, the mesh bags were stored at -20 °C prior to further processing. In the laboratory, grey alder leaf fragments were separated from mineral and other organic particles, and all macroinvertebrates were stored in 70% ethanol. The grey alder leaf material was dried, weighed, ashed and the remaining ash re-weighed to determine the dry mass remaining at sampling (Benfield, 1996). We also accounted for leaching and handling losses (Benfield, 1996). From the data, we calculated leaf litter decomposition rates employing linear and exponential models (Benfield, 1996). The leaf litter decomposition rates were separately calculated for fine and coarse mesh bags; reflecting decomposition due to microbial activity, the combined action of microbes and benthic macroinvertebrates and physical abrasion, respectively.

All macroinvertebrates colonizing the coarse mesh bags were identified to the lowest possible taxonomic level, usually species or genus, but oligochaetes, Hydraenidae and the larvae of some dipteran families could not be classified any further. The primary identification keys were Wallace *et al.* (1990), Arnekleiv (1995), Edington and Hildrew (1995), and Nilsson (1997). From the taxon lists, we calculated the following structural and functional variables: total invertebrate density; taxon richness; the richness of Ephemeroptera, Plecoptera and Trichoptera; Shannon–Wiener diversity; the percentage of rheophile taxa; as well as the relative abundance of invertebrate individuals in the functional feeding groups of shredders (feeding on coarse particulate organic matter), gatherer/collectors (feeding on fine particulate organic matter), grazers/scrapers (feeding on periphyton), and predators, following ASTERICS 4.0.4 (2014) as they provide a link to ecological processes.

In order to test the effect of increased flow as single stressor on periphyton, we used unglazed ceramic tiles (140 × 158 mm) as a substrate for periphyton growth. In order to test the

interacting effects of flow increase and nutrients, we used NDS. The NDS were constructed from inverted clay flowerpot saucers (outside diameter 15.5 cm, height 3 cm, volume: 350 cm³) mounted on unglazed ceramic tiles with silicon (Biggs and Kilroy, 2000). The flowerpot saucers and the tiles were soaked in distilled water for 4 days and dried before the addition of a solution of agar (2%) with added nitrogen (0.5 molar solution of sodium nitrate) and phosphorus (0.05 molar solution of trisodium orthophosphate) or plain agar (2%) for treatment and controls, respectively. Each clay saucer absorbed 30 cm³ of the agar solution in its pores, hence, in total 380 cm³ of solution were added to fill each clay saucer. After the gel reached room temperature, the clay saucers were mounted on ceramic tiles, and sealed with silicon.

On the tiles/NDS we measured total chlorophyll *a* (*Chl a*) concentrations as a proxy of periphyton biomass, and also separately the *Chl a* concentrations of cyanobacteria, green algae, and diatoms using a BenthosTorch, *i.e.* a Pulse Amplitude Modulated fluorimeter developed by BBE Moldaenke GmbH. In Swedish streams, the BenthosTorch has been shown to give similar readings for epilithic *Chl a* as conventional methods (Kahlert and McKie, 2014). In the combined stressor experiments using NDS, the periphyton on the tiles was sampled after the BenthosTorch measurements, and preserved in 0.5% formalin. In the laboratory, algal cells were counted and measured, in order to quantify the biovolume of green algae, diatoms, and cyanobacteria, respectively, and to calculate the number of periphyton taxa that occurred in each sample.

2.4 Experimental design

Each flume was stocked with two pairs of coarse and fine mesh bags, and two tiles (2014) or two NDS (2015; Fig. 1). Tiles were installed in the flumes 13 days before the start of the flow event in order to allow for colonization with periphyton. For the same reason, NDS were installed 3–7 days before the start of the flow event. The position of the NDS's at the left or right-hand side in the flumes was randomized. The mesh bags were exposed for three days in the stream, maximum 20 m from the flumes, to allow colonization of invertebrates. After three days, the mesh bags were moved into the flumes. At each sampling occasion, one randomly selected pair of mesh bags and one tile or NDS was sampled. Sampling was performed twice, once before and once after the flow event (Tab. 1). Drift samples of invertebrates were collected in the beginning of the flow event.

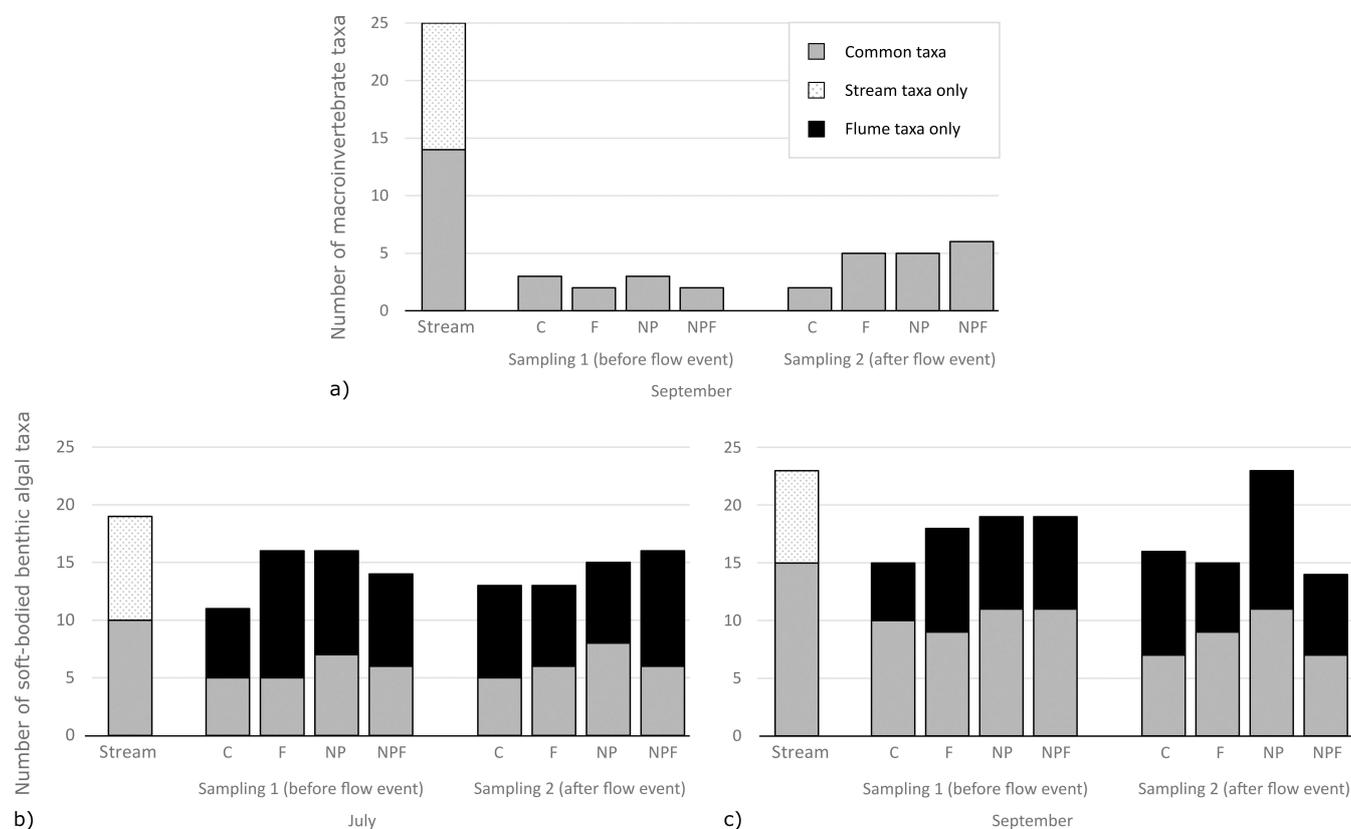


Fig. 2. Number of benthic invertebrate (a) and soft-bodied benthic algal taxa (b = July, c = September) that occurred in both stream and flumes (grey), in the stream only (white), and the flumes only (black). All data are from 2015. Treatments in the flumes are coded as C = control, F = flood, NP = nutrients, NPF = flow and nutrients. Note that sampling 1 occurred before the onset of the flow event.

2.5 Sampling of stream periphyton and macroinvertebrates

During each of the two experimental rounds performed in 2015, samples of soft-bodied benthic algae (=algae including cyanobacteria attached to the river bottom or in close contact on or within patches of attached aquatic plants, but excluding diatoms) were taken 5–10 m from where the flumes were located in Sagelva, according to European standard procedures (EN 15708:2009). All macroscopically visible benthic algae were collected and samples were stored separately in vials for species determination. In addition, microscopic algae were collected from ten cobbles/stones with diameters ranging between approximately 10 and 20 cm. An area of about 8×8 cm from the upper side of each cobble/stone was brushed with a toothbrush to transfer the algae into a beaker containing approximately 1 L of river water from which a subsample was taken. All samples were preserved with a few drops of formaldehyde to a final concentration of approximately 0.5%. The preserved benthic algae samples were later examined under a microscope and all non-diatom algae identified to species, wherever possible. The primary identification keys used were Komarek and Anagnostidis (2007), Gutowski and Förster (2009), John *et al.* (2011) and Komarek (2013). For some genera of filamentous green algae whose vegetative forms cannot be determined to species level (*e.g.* *Spirogyra* Link or *Mougeotia* C. Agardh) categories based mainly on filament width were used (see Schneider and Lindstrøm (2009,

2011) for further details). In order to compare the species composition of benthic algae in the stream with the flumes, presence–absence data had to be used, because the semiquantitative sampling of taxa from a stream greatly differs from the quantitative sampling and enumeration that is possible on tiles/NDS.

Macroinvertebrates from the Sagelva stream were collected in September 2015, 10–20 m from the flumes, from the same location where the mesh bags were pre-exposed prior to each experimental round. We performed qualitative sampling using kick-net (mesh size 500 μ m) and three 1-min samples covering a total area of approximately 0.9 m². The samples were processed in the laboratory, the macroinvertebrate community was determined to the lowest possible taxonomic level and recorded as present/absent.

2.6 Data analysis

We used the software package R 3.3.2 (R Core Team, 2016) including the packages *vegan* and *lmerTest* for data analysis (Oksanen *et al.*, 2013; Kuznetsova *et al.*, 2016). Data were log-transformed where necessary to improve normality and heteroscedasticity (visual inspection of histograms). We performed ANOVAs on linear mixed models using “experimental round” as random factor to analyze the individual and combined effects of an increase in nutrients and water flow on invertebrates, leaf litter degradation and periphyton algae.

Table 2. Summary (*p*-values) of ANOVAS comparing leaf decay and invertebrate parameters in mesh bags, as well as periphyton parameters on tiles between flow increase and control treatments. Significant effects ($p < 0.05$) of flow are marked in bold, *p*-values < 0.1 are marked in italics.

Dependent variable	Flow increase	Ranking
<i>Leaf litter decomposition rate</i>		
Coarse mesh bags (k)	0.099	<i>Control < flood</i>
Fine mesh bags (k)	0.204	
Coarse-fine (k)	0.925	
<i>Drift</i>		
Number of drifting individuals	0.086	<i>Control < flood</i>
<i>Invertebrates in coarse mesh bags</i>		
Total invertebrate density	0.162	
Taxon richness	0.433	
EPT richness	0.273	
Shannon–Wiener diversity	0.286	
% Rheophile	0.354	
Proportion of grazers/scrapers	0.180	
Proportion of shredders	0.678	
Proportion of gathering collectors	0.548	
Proportion of predators	0.295	
<i>Periphyton</i>		
Total <i>Chl a</i>	0.108	
Cyanobacteria	0.026*	Flood < control
Diatoms	0.177	
Green algae	0.009*	Flood < control

3 Results

3.1 Differences between stream and flumes

Water temperature in the Sagelva stream during the experiments in 2015 was $15 \pm 1^\circ\text{C}$ (min 12°C , max 18°C ; data not shown). In both experimental rounds, the water temperature in the flumes followed the same pattern as in the stream, but was slightly higher (paired *t*-test, exp. round 1: $+0.20^\circ\text{C}$ ($p < 0.001$), exp. round 2: $+0.04^\circ\text{C}$ ($p < 0.001$)).

We found 25 taxa of benthic invertebrates in the Sagelva stream in September 2015 (Tab. S1). In contrast, there occurred on average 3.5 ± 1.5 macroinvertebrate taxa in a coarse mesh bag in the flumes in 2015. In total across both experiments and all treatments in 2015, 14 taxa were found in the mesh bags in the flumes. All of these taxa were also found in the September stream sample (Fig. 2, Tab. S1). Particularly Plecoptera, but also Ephemeroptera and Trichoptera were more species rich in the stream than in the flumes (Tab. S1). The most abundant taxa in the flumes were chironomids, the mayflies *Baetis rhodani* (Pictet, 1843) and *Ephemerella ignita* (Poda, 1761) and the wandering snail *Radix balthica* (Linnaeus, 1758) (Tab. S1). Generally, the number of individuals in the flumes was higher in July than in September (Tab. S1). The taxa that were only present in the stream included the mayfly *Heptagenia sulphurea* (Müller, 1776), the stoneflies *Isoperla grammatica* (Poda, 1761), *Dinocras*

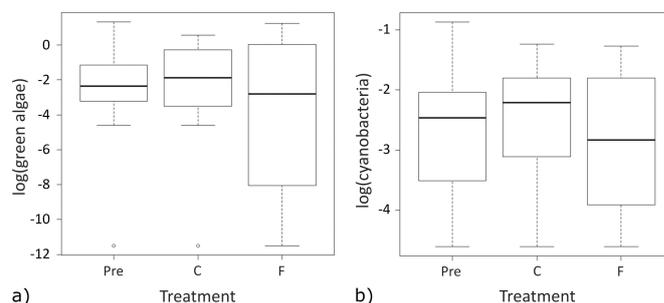


Fig. 3. Biomass of (a) green algae and (b) cyanobacteria (in $\mu\text{g Chl a cm}^{-2}$) before the flow event (=day 0 of the experiment; indicated as “Pre”), and after the flood event (day 7 of the experiment) in control (C) and flow treatments (F). The box displays the first and third quartile, the band inside the box represents the median; the whiskers denote minimum and maximum; data more than 1.5 interquartile range below the first or above the third quartile, were treated as outliers (o).

cephalotes (Curtis, 1827), *Perlodidae* and *Protonemura meyeri* (Pictet, 1841), the caddisflies *Hydropsyche* sp. and *Hydroptila* sp., the riffle beetle *Elmis aenea* (Müller, 1806) and the dagger fly *Empididae*.

For soft-bodied benthic algae, generally more taxa occurred in September 2015 than in July, and fewer taxa in the flumes than in the stream (flumes: 16 ± 3 taxa; stream: 21 ± 2 taxa; Fig. 2). Across both experiments and samplings, 9 ± 3 taxa were shared between flumes and stream, 9 ± 1 taxa occurred only in the stream, and 8 ± 2 taxa occurred only in the flumes (Fig. 2). Among the most common taxa that occurred both in the stream and the flumes were *Closterium* sp., *Cosmarium* sp., narrow *Mougeotia* filaments, and *Ulothrix* sp. (Tab. S2 in the Supplementary Material). The most common taxa that occurred exclusively in the stream were epiphytic taxa like *Chamaesiphon rostafinskii* (Hansgirg, 1887), broad *Oedogonium* filaments, and the red algae *Audouinella chalybaea* ((Roth, 1806) Bory, 1823) and *Batrachospermum* sp. The most common taxa that occurred in the flumes only were small coccoid taxa like *Aphanocapsa* sp., *Chroococcus* sp. or *Euastrum* sp.

3.2 Flow increase as single stressor

The flow event decreased the biomass of cyanobacteria and green algae (Tab. 2; Fig. 3), but had no significant effect on any of the other response variables. However, leaf litter degradation in the coarse mesh bags tended to be enhanced in the flow treatment, but that trend was insignificant. Also, the number of drifting individuals tended to be higher in the high flow treatment, but that trend was also insignificant (Tab. 2). Compared to pre-flood conditions, the biomass of cyanobacteria and green algae increased slightly in the control treatments, but decreased in the flow treatments (Fig. 3).

3.3 Combined effects of flow increase and nutrient addition

Before the onset of the flow treatment (day 0 of the experiment), no significant effects were observed between

Table 3. Summary (*p*-values) of ANOVAS comparing leaf decay and invertebrate parameters in mesh bag samples, as well as periphyton parameters, on nutrient diffusing substrates between nutrient and flow treatments; samples were taken 5 (round 1) and 4 (round 2) days after the cessation of the three days long flow event; significant effects (*p* < 0.05) are marked in bold, *p*-values < 0.1 are marked in italics.

Dependent variable	Flow increase	Ranking	Nutrients	Ranking	Flow x nutrients	Type of interaction
Leaf litter decomposition rate						
Coarse mesh bags (k)	<i>0.062</i>	<i>Control < flood</i>	0.295		0.175	
Fine mesh bags (k)	0.818		0.034	Control < nutrients	<i>0.058</i>	
Coarse-fine (k)	0.221		0.580		<i>0.052</i>	
Drift						
Number of drifting individuals	0.311		0.006	Control < nutrients	0.507	
Invertebrates in coarse mesh bags						
Total invertebrate density	<i>0.094</i>	<i>Control < flood</i>	0.353		0.774	
Taxon richness	0.274		0.778		0.981	
EPT richness	0.422		0.880		0.880	
Shannon-Wiener diversity	0.361		0.999		0.896	
% rheophile	0.955		<i>0.074</i>	<i>Nutrients < control</i>	0.264	
Proportion of grazers/scrapers	0.981		0.747		0.973	
Proportion of shredders	0.883		0.027	Nutrients < control	0.704	
Proportion of gathering collectors	0.317		0.264		0.703	
Proportion of predators	0.535		0.044	Control < nutrients	0.480	
Periphyton						
Total Chl a	0.002	Control < flood	<0.001	Control < nutrients	0.014	Antagonistic
Cyanobacteria (Chl a)	<i>0.076</i>	<i>Control < flood</i>	0.003	Control < nutrients	<0.001	Antagonistic
Diatoms (Chl a)	0.001	Control < flood	<0.001	Control < nutrients	<0.001	Antagonistic
Green algae (Chl a)	<i>0.093</i>	<i>Control < flood</i>	<0.001	Control < nutrients	0.523	
Biovolume Cyanobacteria	0.349		<i>0.083</i>	<i>Control < nutrients</i>	0.610	
Biovolume green algae	0.562		0.145		0.540	
Biovolume diatoms	0.658		<i>0.054</i>	<i>Control < nutrients</i>	0.116	
Total biovolume	0.819		0.114		0.305	
Taxon richness	0.384		0.793		0.740	

nutrient and control treatments in any of the response variables. However, degradation of leaf litter by macro-invertebrates (coarse-fine mesh bags) and total invertebrate density were marginally higher in the nutrient treatments (Tab. S3 in the Supplementary Material).

In the samples taken after the flow event, nutrient addition increased the biomass of all algal groups and of total *Chl a*, increased the leaf litter degradation rate in the fine mesh bags, increased the number of drifting individuals and the relative amount of predators, but decreased the relative amount of shredders (Tab. 3). The flow event increased total *Chl a*, and specifically the biomass of diatoms (Tab. 3). No interactions between flow and nutrient levels occurred for invertebrate variables, but antagonistic interactions were observed for cyanobacteria, diatoms and total *Chl a* (Tab. 3; Fig. 4).

4 Discussion

4.1 Differences in invertebrate and periphyton composition between stream and flumes

Our results support hypothesis 1, which stated that there would be fewer taxa in the flumes than in the stream. This was true for macroinvertebrates and soft-bodied benthic algae, although the difference in species number was more pronounced for macroinvertebrates (Fig. 2). Also taxon identity differed between stream and flumes, but in this case

the difference was more pronounced for soft-bodied benthic algae (Fig. 2). In the macroinvertebrate assemblages there occurred taxa which exclusively were found in the stream (“stream only”), but all taxa that were found in the flumes also occurred in the stream (we found no “flumes only” taxa). In contrast, the benthic algal assemblages contained “flumes only” as well as “stream only” taxa. Our flumes were constructed as through-flow (*i.e.* without recirculating water) with rapid water exchange being provided directly from the stream, through a comparatively small tank which was necessary to enable constant water supply to all flumes. For these reasons, major differences in water physico-chemistry between flumes and stream are highly unlikely to have occurred. We monitored water temperature, and although the difference between stream and flumes indeed was significant, the difference of just 0.2 and 0.04 °C is so small that it is unlikely to have caused the observed difference in macro-invertebrate and benthic algal assemblages between stream and flumes.

The red algae *Audouinella* sp. and *Batrachospermum* sp. were absent from the flumes, likely due to the short duration of the experiment which prohibited the successful establishment of these slowly growing taxa on the tiles/NDS. For similar reasons, small epiphytes such as *Chamaesiphon* sp. also were absent from the flumes, likely because their successful establishment obviously only can occur after suitable substrate algae have colonized the flumes. Obviously, colonization by

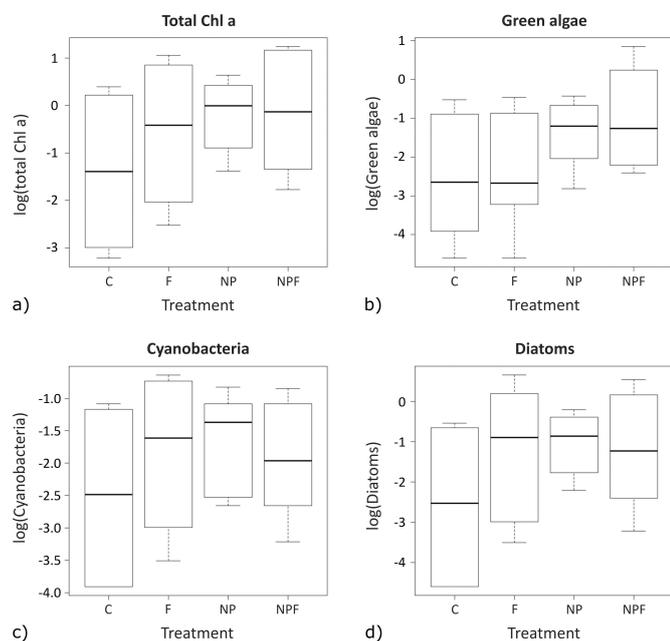


Fig. 4. (a) Total *Chl a*, as well as (b) biomass of green algae, (c) cyanobacteria and (d) diatoms (all in $\mu\text{g Chl a cm}^{-2}$) in control (C) and flow (F), nutrient (NP) and combined nutrient-flow (NPF) treatments. The box displays the first and third quartile, the band inside the box represents the median; the whiskers denote minimum and maximum; data more than 1.5 interquartile range below the first or above the third quartile, were treated as outliers (o).

epiphytic algae would have required more time than our experiment lasted. In contrast, small coccoid and fast growing algae preferably occurred in the flumes. This may be explained by the classical concept of C-S-R life history strategies (Grime, 1977), where small, rapidly growing “pioneer” or “ruderal” species succeed in disturbed (or new) habitats with moderate to high nutrient supply (Biggs *et al.*, 1998b), a type of habitat which our previously uncolonized NDS likely represented.

With respect to macroinvertebrates, both the stream sample and the mesh bag assemblages from the flumes were dominated by generally abundant taxa, such as chironomids and the mayfly *Baetis* sp. However, particularly Plecoptera, but also Ephemeroptera and Trichoptera were more species rich in the stream than in the flumes, and many taxa that occurred in the stream were not found in the flumes. This may be due to several reasons: (i) insufficient numbers of a species were present in the stream during the time when the mesh bags were pre-exposed such that these species were rather unlikely to colonize the mesh bags; (ii) short duration of pre-exposure of the meshbags in the stream. Biasi *et al.* (2013) found that the colonization of leaf litter bags considerably changed within 22 days of exposure in a stream; our 3–4 days may therefore have been too short to capture the entire macroinvertebrate assemblage present in the stream. However, a longer pre-exposure in the stream was undesired because the leaf litter would have been degraded already before the bags were exposed in the flumes. (iii) Leaf litter bags may not be colonized by taxa which prefer hard substrates; the importance of substratum type on macroinvertebrate density and composition has for example been highlighted by Dumnicka

et al. (2007). Many of these effects may possibly have been avoided by assisting the colonization of the flumes by adding a standard load of invertebrates obtained from the stream by kick-net sampling (as *e.g.* done by Matthaei *et al.*, 2010).

Flume experiments are a common means to study the effect of stressors on stream ecosystems (*e.g.* Matthaei *et al.*, 2010; Oldmeadow *et al.*, 2010; Elbrecht *et al.*, 2016), and conclusions with respect to structural and functional responses of stream ecosystems to stressors are frequently drawn from flume experiments (*e.g.* Ledger *et al.*, 2011; Villeneuve *et al.*, 2011; Ceola *et al.*, 2013). However, biodiversity affects stream functioning through interspecific facilitation (Cardinale *et al.*, 2002), and a higher biodiversity is generally assumed to stabilize ecosystem functions in response to disturbances and variation in abiotic conditions (Hooper *et al.*, 2005). However, despite their widespread use in aquatic environments, few publications using flume experiments report the natural diversity of the analyzed organism groups in the nearby lotic ecosystem, and compared it with the diversity in the experimental flumes (but see Ledger *et al.*, 2009). Although we do admit that the number of macroinvertebrate taxa in our flumes may have been enhanced, for example by adding standard loads from kick-net sampling in the Sagelva stream, our experimental design generally is similar to other flume experiments (*e.g.* Villeneuve *et al.*, 2011; Cañedo-Argüelles *et al.*, 2014). We therefore believe that diversity in experimental flumes maybe generally lower than in natural stream ecosystems, such as was the case in our experiments. Also Ledger *et al.* (2009) reported less taxa in the flumes than in the stream. This should mean that many flume experiments may lack “ecological realism” (Ledger *et al.*, 2009). Experiments are necessary in order to deduce causal relationships, since these cannot be inferred from correlative field data. However, we agree with Schindler (1998) that small scale experiments may give highly replicable, but spurious, answers. It is therefore necessary to combine causal evidence from experiments with correlative evidence from field data. Only if both agree with each other, results from flume experiments may be assumed to be applicable in the “real world” with some confidence.

4.2 Effects of flow increase and nutrients on leaf litter degradation, invertebrates and periphyton

Hypothesis 2, which stated that the flow event would decrease benthic algae and macroinvertebrate biomass and diversity, was accepted for periphyton but rejected for macroinvertebrates. Drift of macroinvertebrates increased at the beginning of the flow event, although this effect only was marginally significant (Tab. 2). This meets expectations, because drift is a common response of macroinvertebrates to increased flow (Robinson *et al.*, 2004). An increase of flow from 2.9 to 6.9 cm s^{-1} in the 2014 experiments decreased the biomass of cyanobacteria and green algae, but had no effect on any of the invertebrate response variables. This was not surprising, because macroinvertebrates and algae were collected from a stream next to our flumes. Although we do not have data on flow velocity from this stream, our flumes were constructed to have a similar flow velocity as the stream (based on visual inspection). The climate in Norway is

notoriously variable, and frequent changes in stream discharge are common (www.nve.no). More serious spates in the stream Sagelva most probably comprise flow velocities well above 7 cm s^{-1} , *i.e.* faster than what was possible to achieve in our experiments. It is reasonable to assume that most of the macroinvertebrate species we collected in the stream were adjusted to variations in flow velocity, and reacted by small-scale movements, *e.g.* alteration of body posture, to the increased flow velocity (Lancaster, 1999). The decrease in the biomass of benthic algae may be due to abrasion or scouring (Francoeur and Biggs, 2006). Overall, our experiments showed that an increase in flow velocity from 2.9 to 6.9 cm s^{-1} may lead to removal of some algae, drift of some invertebrates, but most macroinvertebrates will be able to cope with the increased flow by, for example, adjustments of body posture. It is, however, likely, that these effects in “real” stream ecosystems will not be long-lasting, because recolonization will commonly be fast (Schneider and Petrin, 2017).

An increase of flow from 1.3 to 2.8 cm s^{-1} in the 2015 experiments lead to an increase in the biomass of diatoms and total *Chl a*, but affected no other response variables (Tab. 3). This seems at first glance, contradictory to the results obtained in the 2014 experiments, where we observed a decrease in the biomass of cyanobacteria and green algae when flow was increased from 2.9 to 6.9 cm s^{-1} . It is, however, a well-known phenomenon that mucilaginous algae, such as diatoms, may increase with flow velocity due to increased rates of nutrient transfer into the assemblages, while filamentous forms, such as green algae decrease due to scouring (Biggs *et al.*, 1998a). In general, our results indicate an increase in flow velocity in the “lower” range may lead to an increased algal biomass, but that a further increase of flow velocity may lead to a decrease of algal biomass. This matches well with field data from Norway (Schneider, 2015), showing that a flood event may decrease algal cover in a headwater stream (where flow velocities during floods may be high), but a higher August flow actually increased green algal cover in a lake outlet (where changes in flow velocities generally are more buffered than in headwater streams).

Hypothesis 3, which stated that nutrients would increase the biomass of benthic algae and decrease the rate of leaf litter breakdown, was accepted for benthic algae but rejected for leaf litter breakdown. Nutrients did not have an effect on any of the periphyton response variables at day 0 (Tab. 3), likely because sampling after only 3–7 days of exposure of the NDS was too early to show an effect. However, one week later the nutrient treatment was associated with an increased biomass of cyanobacteria, diatoms and green algae (Tab. 3). Increased benthic algal growth at enhanced nutrient supply is a well-known phenomenon (Francoeur *et al.*, 1999). We found an increased rate of microbial degradation (fine mesh bags) in the nutrient treatments (Tab. 3). This fits well with early studies that have shown a faster breakdown in nutrient-rich systems (Webster and Benfield, 1986) and with experimental addition of nutrients (Robinson and Gessner, 2000). This was generally attributed to an increased availability of nitrogen to microbiota (Webster and Benfield, 1986). Also our study system was relatively poor in nitrogen (total nitrogen $235\text{--}370 \mu\text{g N l}^{-1}$), such that a faster degradation of leaf litter in the nutrient enhanced treatment seems plausible.

We observed antagonistic interactions between nutrients and flow for total *Chl a*, diatoms and cyanobacteria, *i.e.* the combined effect of flow and nutrients was less than the sum of both individual effects (Piggott *et al.*, 2015). As a single stressor, nutrients may lead to an increase in the biomass of benthic algae, and the same is true for an increased flow velocity in the range from 1.3 to 2.8 due to an improved transfer of nutrients into algal patches (see above). However, an increased biomass also is more susceptible to scouring such that the combined effect of nutrients and a flow increase is less than the sum of both individual effects. Hypothesis 4, which stated that a flow event in the high nutrient treatment would reduce benthic algae more than in the low nutrient treatment, was therefore partly accepted.

We observed fewer effects of increased flow on invertebrates than Elbrecht *et al.* (2016) or Matthaei *et al.* (2010) observed in their experiments testing reduced flows. We suggest that this may be explained because stream invertebrates in Norway may be adapted to frequent changes in stream flow caused by weather conditions. A long-lasting decrease in flow velocity, as tested by Elbrecht *et al.* (2016) and Matthaei *et al.* (2010) represents an entirely different stressor, entailing decreased oxygen concentrations, increased temperature and siltation. Experiments simulating an increase in flow are therefore not directly comparable with experiments simulating a decrease in flow.

Supplementary Material

Tables S1 to S3.

The Supplementary Material is available at <http://www.kmae-journal.org/10.1051/kmae/2017041>.

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