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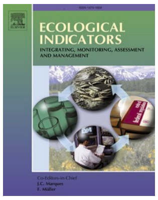

Original Articles

Responses of multiple structural and functional indicators along three contrasting disturbance gradients

Amélie Truchy [a,*], Ryan A. Sponseller [b], Frauke Ecke [c], David G. Angeler [a,d], Maria Kahlert [a], Mirco Bundschuh [a,e], Richard K. Johnson [a], Brendan G. McKie [a]

[a] *Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden*
[b] *Department of Ecology and Environmental Sciences, Umeå University, Umeå, Sweden*
[c] *Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden*
[d] *School of Natural Resource, University of Nebraska – Lincoln, Lincoln, NE, USA*
[e] *Institute for Environmental Sciences, University of Koblenz-Landau, Fortstraße 7, 76829 Landau, Germany*

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ABSTRACT

Ecosystem functioning and community structure are recognized as key components of ecosystem integrity, but comprehensive, standardized studies of the responses of both structural and functional indicators to different types of anthropogenic pressures remain rare. Consequently, we lack an empirical basis for (i) identifying when monitoring ecosystem structure alone misses important changes in ecosystem functioning, (ii) recommending sets of structural and functional metrics best suited for detecting ecological change driven by different anthropogenic pressures, and (iii) understanding the cumulative effects of multiple, co-occurring stressors on structure and function. We investigated variation in community structure and ecosystem functioning of stream ecosystems along three gradients (10–16 independent stream sites each) of increasing impact arising from agriculture, forestry and river regulation for hydropower, respectively. For each stream, we quantified variation in (i) the abiotic environment, (ii) community composition of four organism groups and (iii) three basal ecosystem processes underpinning carbon and nutrient cycling in streams. We assessed the responsiveness of multiple biodiversity, community structure and ecosystem functioning indicators based on variance explained and effect size metrics. Along a gradient of increasing agricultural impact, diatoms and fish were the most responsive groups overall, but significant variation was detected in at least one aspect of community composition, abundance and/or biodiversity of every organism group . In contrast, most of our functional metrics did not vary significantly along the agricultural gradient, possibly due to contrasting, antagonistic effects of increasing nutrient concentrations and turbidity on ecosystem process rates. The exception was detritivore-mediated litter decomposition which increased up to moderate levels of nutrient. Impacts of river regulation were most marked for diatoms, which were responsive to both increasingly frequent hydropeaking and to increasing seasonal river regulation. Among functional indicators, both litter decomposition and algal biomass accrual declined significantly with increasing hydropeaking. Few structural or functional metrics varied with forest management, with macroinvertebrate diversity increasing along the forestry gradient, as did algal and fungal biomass accrual. Together, these findings highlight the challenges of making inferences about the impacts of anthropogenic disturbances at the ecosystem level based on community data alone, and pinpoint the need to identify optimal sets of functional and structural indicators best suited for detecting ecological changes associated with different human activities.

1. Introduction

Freshwater habitats are among the most highly exploited worldwide, and are impacted by multiple human activities (Malmqvist and Rundle, 2002; Vörösmarty et al., 2010). Previous research has documented extensive impacts of agriculture, forestry, and hydropower on not only the diversity and composition of organism groups (Johnson and Almlöf, 2016; McKie and Cranston, 2001), but also on the ecosystem processes they regulate (Matthaei et al., 2010; McKie and Malmqvist, 2009). However, few studies have compared the impacts of multiple pressures

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* Corresponding author.
E-mail address: amelie.truchy@slu.se (A. Truchy).

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using standardized sets of functional and structural indicators (e.g. Wagenhoff et al. (2017)). This research gap limits our capacity to (i) assess whether monitoring of ecosystem structure alone misses important functional changes associated with different anthropogenic pressures, (ii) identify sets of structural and functional indicators suited for detecting ecological change driven by different types of human impact, and (iii) detect cumulative effects of multiple, co-occurring stressors on structure and function.

Despite increasing calls for better integration of functional indicators into legislative frameworks (Birk et al., 2012; WFD, 2000; Aron et al., 2017), most routine monitoring of stressor impacts continues to focus on metrics of community structure rather than ecosystem processes. In part, this reflects the relatively short history of criteria development for assessing functional integrity based on quantification of ecosystem processes (e.g. Chauvet et al., 2016), in comparison with the long history of assessment of structural integrity based on monitoring of organism groups (e.g. Hering et al., 2006b; Wright et al., 2000). Additionally, structural indicators are often posited to respond more rapidly to environmental change than ecosystem functioning (Palmer et al., 2005; Schindler, 1990), based on the ability of stress-tolerant biota to maintain ecosystem functioning under disturbance (Vinebrooke et al., 2004; Yachi and Loreau, 1999). However, there are cases where ecosystem functioning changes without concomitant community changes (McKie and Malmqvist, 2009), highlighting the risks in inferring functional integrity based on community structure alone.

After decades of research on structural indicators for biomonitoring, it is now possible to make recommendations for which sets of community metrics are most suited for monitoring different types of disturbances (Hering et al., 2006b). It might in some cases be possible to nominate a single ecosystem process as a proxy for ecosystem function *per se* (e.g. Gessner and Chauvet, 2002; Venkiteswaran et al., 2008), especially when the environmental gradient is dominated by one abiotic variable which has effects on multiple food web components (Von Schiller et al., 2008). However, other studies have found divergent responses for different functional indicators (Young and Collier, 2009; Frainer et al., 2017), reflecting (i) differences in the environmental sensitivities of organisms underpinning different ecosystem processes (Bradford et al., 2014), and (ii) complex antagonistic/synergistic interactions among co-occurring stressors, which constrain or enhance responses of individual functional indicators (Crain et al., 2008; Jackson et al., 2016). Further, while functional responses to some human impacts (e.g. nutrient enrichment) are reasonably well documented, others (e.g. river regulation) remain poorly understood. These knowledge gaps hinder the identification of particular sets of structural and functional indicators best suited for assessing different types human impacts (Matthaei et al., 2010; Townsend et al., 2008).

Here, we investigate variation in community structure and ecosystem functioning along gradients of increasing impact from agriculture, forestry, and river regulation associated with hydropower dams. These impacts were characterized first at the whole catchment scale for each stream site, based on the percentage of agriculture, forestry and volume of water regulated in the catchment respectively. At each stream site, we then gathered data on (i) local-scale abiotic variables (e.g. nutrients, pH, flow velocity), (ii) community composition of four organism groups (benthic diatoms, macrophytes, benthic invertebrates and fish), and (iii) three ecosystem processes (algal biomass accrual, fungal biomass accrual and litter decomposition rates). We used these data to assess the responsiveness of these indicators along our impact gradients in terms of not only their statistical significance, but also variance explained (R^2) and effect sizes, to gain insight into which indicator sets are most appropriate for assessing impacts of different pressures on both ecosystem structure and function.

We expected a general increase in ecosystem process along the agricultural gradient in response to the bottom up stimulation of microbial and algal activity associated with nutrient enrichment (Young and Huryn, 1999; Gulis and Suberkropp, 2003) (functional **H1**). We hypothesized that algal biomass accrual and litter decomposition would decline strongly in response to increasing river regulation, reflecting the vulnerability of algal and microbial biofilms to the episodes of water restriction (e.g. Timoner et al. (2012); Truchy et al. (2020)) (functional **H2**). The main factors varying along our forestry gradient, including an increase in conifer cover and forest ditching with associated sediment transport (Ecke, 2009, Stenberg et al., 2015) were expected to suppress all ecosystem processes, in line with previous results (e.g. Kominoski et al., 2011; Frainer and McKie, 2021) (functional **H3**). Finally, our predictions for structural indicators were based on those arising from the analysis of an extensive European scale database, produced to support the EU water framework directive (Hering et al., 2006a, Hering et al., 2013). As predicted by Hering et al. (2013), we expected that all four organism groups would respond to nutrient enrichment (the primary variable changing along our agricultural gradient), whereas river regulation would have the strongest impacts on fish and benthic invertebrates, while diatoms and benthic invertebrates were expected to respond to the diffuse changes associated with increased forestry (structural **H4**).

2. Materials and methods

2.1. Study systems: Overview

Biota, comprising benthic diatoms, macrophytes, benthic invertebrates, and fish, as well as ecosystem processes and environmental variables, were quantified in 36 second to third order stream reaches across three regions in Sweden (Fig. 1; Table 1). Stream sites in each region ranged from forested streams with little human impact to those heavily impacted by human activities. All study reaches (circa 100 m long) had hard substrates dominated by gravel and cobbles, and a riparian strip composed of predominantly woody vegetation that shaded the stream channel, although the lateral extent of riparian vegetation varied e.g. between streams flowing through agricultural fields compared with those through forest. Impacted sites on the river regulation gradient were always sampled downstream of a dam (Table 1), though it was generally not possible to access the stream immediately downstream of the dam wall owing to safety and access regulations.

2.2. Characterizing the gradients at the catchment level

To characterize agricultural pressures, we used catchment landcover classified as agriculture obtained from the Swedish Landcover Map 2004, nutrient concentrations (nitrogen, phosphorus, nitrates, phosphates), and a semi-quantitative estimate of pesticide use in the catchments. Estimates of pesticide use were based on the yearly interviews conducted by the Centre for Chemical Pesticides (CKB), reports from Statistics Sweden (SCB, 2011), and area devoted to cereal productions (Swedish Board of Agriculture, 2012) (Table S1.1).

The extent of river regulation was characterized from modelled hydrology and data from the Värmland county administrative board (Hedenskog et al., 2015). Deviation in discharge attributable to river regulation, the volume of water regulated (i.e. the difference between the current and natural flow regime based on daily averages), and the proportion of runoff stored in upstream reservoirs were obtained using the Swedish HYPE (HYdrological Predictions for the Environment) model available from the Swedish Meteorological and Hydrological Institute (SMHI, http://vattenwebb.smhi.se/). Using discharge data from the year of sampling and one year prior (from SMHI), we applied the Dundee Hydrological Regime Assessment Method (DHRAM, Black et al. (2005)) to calculate 19 parameters characterizing the extent of hydrological alteration for each stream (Table S1.1). In addition, data on the maintenance of simplified, channelized habitats via ongoing removals of dead wood and vegetation was also obtained from the Värmland county administrative board.

Forestry pressures were quantified using catchment landcover

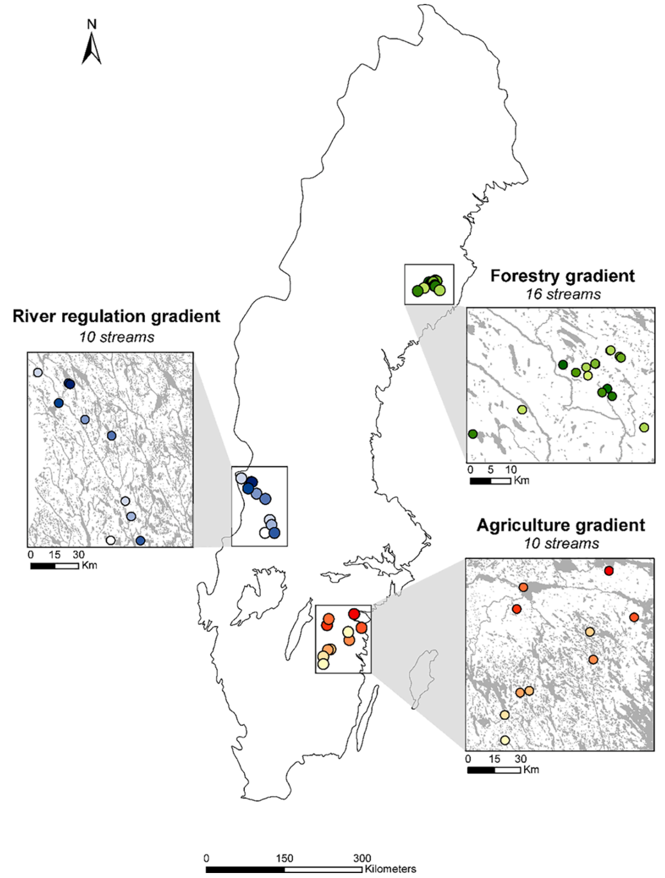

Fig. 1. Location of the 36 stream sites across Sweden representing three anthropogenic gradients. The forestry gradient comprised 16 streams (green circles) while the river regulation and agricultural gradients each consisted of 10 streams (blue and orange circles, respectively). Along each gradient, a color ramp indicates the strength of impact with the less impacted streams being represented by light-colored symbols while the most heavily impacted stream sites are dark colored, based on PC scores (PC1 for both the agricultural and river regulation gradient, PC2 for the forestry gradient). The main variables increasing along the agricultural gradient included the proportion of agriculture in the catchment, total nitrogen and water turbidity, while dissolved oxygen concentrations declined (Fig. S1.1a). The river regulation gradient was characterized by an increasing deviation in water volume from the natural flow regime in line with increasing hydropeaking and decreasing TOC (Fig. S1.1b). The forestry gradient was characterized by an increasing proportion of catchment clear-cutting and ditching, a decreasing proportion of coniferous trees in younger forest (Fig. S1.1c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

classified as coniferous forest, clear-cuts (logging records since 2001; Swedish Forestry Agency) as well as stand age and tree volume (SLU Forest Map, Department of Forest Resource Management, Swedish University of Agricultural Sciences, 2010). Unfortunately, the two most impacted stream sites from this gradient (~60% and 70% of clear-cuts in the catchment) had insufficient water to sample following the dry summer of 2013, and were dropped from all analyses.

Swedish catchments are characterized by extensive ditching networks, constructed over previous decades-centuries to drain forest and agricultural land, altering water chemistry, sediment loads and the hydrology of the receiving stream (Hasselquist et al., 2018). We digitized ditches in each of our stream catchments based on a Digital Elevation Model (DEM, 2 m grid, from Lantmäteriet) using the Hillshade tool (ArcGIS, ESRI ArcMap, 10.2) and setting the sun angle at 90° and an altitude of 20 m a.s.L. The stream channel was distinguished from the ditch network by controlling its position using topographic GIS layers. The extent of ditching within a catchment was quantified as the cumulative length of ditches in the catchment divided by the stream length.

2.3. Measurements of local abiotic variables

A common set of local environmental variables was measured at each study reach, including stream depth and width, slope, canopy cover (estimated using a Leaf Area Index (LAI) canopy analyzer (LI-CORE® LAI-2000, Lincoln, Nebraska, USA)), and flow velocity (MiniAir 20 with a Mini sensor 22 m 5 m/s, Schiltknecht, Gossau, Switzerland). Stream temperature was recorded continuously during each study period using “SmartButton” data loggers SL5x (Signatrol, Tewkesbury, UK), while pH, turbidity, saturation in dissolved oxygen and conductivity were assessed every third week (four occasions per stream) using a MANTA multiprobe (Eureka Environmental Engineering, Austin, Texas, USA). Two 0.25 L filtered (0.7 μm glass fiber paper, Whatman, Brentford, UK) water samples were collected at each stream site to assess dissolved organic carbon (DOC), total organic carbon (TOC) and other chemical variables, including concentrations of total nitrogen and total phosphorus (Table 1 & Table S1.1). Local abiotic data collected during the whole study period were summarized as means.

2.4. Biotic community sampling and identification procedures

Each organism group was sampled once per region, with sampling for each group always completed within 5 days per region.

Benthic diatoms were collected during summer (end of July-August), identified and enumerated following the standard national protocol (SS-EN-13946; SS–EN 14407; CEN (2004)). Sampling was undertaken by scraping biofilm from the upper surface of five cobbles (10–25 cm diameter), which were then pooled per stream. The samples were stored in dark bottles and preserved with Lugol’s iodine solution before identification and counting. At least 400 diatom valves from each sample were counted and identified mainly to species level (Table S2.1) under the microscope. Biodiversity metrics were calculated and used in our statistical models.

Macrophytes, comprising aquatic vascular plants, bryophytes and macroalgae, were sampled during late summer (August) following the Swedish EPA’s protocol (Naturvårdsverket, 2003). In each stream, we sampled 100 quadrats along six to 10 transects, depending on stream width. Along each transect, 25 × 25 cm quadrats were placed side by side from one bank to the other. The presence of macrophytes was recorded in each quadrat using an aquascope. Subsamples were taken when necessary for species validation in the laboratory. Relative frequencies of species in the quadrats were calculated along with biodiversity metrics and used in our statistical models.

Benthic invertebrates were sampled during the autumn (October) according to European and Swedish standards (SS-EN 10870:2012; Naturvårdsverket, 2010). In brief, five samples per stream were taken using standardized kick sampling (0.5-mm mesh size) in riffle habitat. The bottom substratum was disturbed for 60 s along a 1 m long stretch and invertebrates collected. Samples were preserved in 70% ethanol before sorting and identification according to the Swedish bioassessment standards. Invertebrate abundances and biodiversity metrics were used in further statistical models.

Fish assemblages were sampled by electro-fishing in late summer-early autumn (end of August-September) according to the European standard method (SS-EN 14011: 2006). A 20–50 m long reach was electro-fished using a bank-based generator coupled to a single hand-held anode. The total area sampled depended on stream width, with longer reaches sampled in smaller streams. The number of passes at each stream site varied between one and three. Fish were identified to species. Species densities (number of individuals m^{-2}) were estimated accounting for the probability of catch at each stream site and used in further statistical models, along with biodiversity metrics.

Table 1

Environmental variables describing the anthropogenic gradients measured in each of the 36 streams. Although all pressures were present along each of the gradients, one was dominant in each region (i.e. agriculture or river regulation or forestry). The agricultural gradient represented a gradual increase in the proportion of agricultural land in the catchment as well as an increasing ditching index and nutrient concentrations (TN: Total nitrogen). The main factors varying along the river regulation gradient include the deviation in water volume from the natural flow regime, along with the number of flow rises and the date of minimum flow. Streams exhibiting strong impacts of forestry are located in the vicinity of clear-cuts and are characterized by higher ditching index, percentage of clear-cuts in the catchment and younger forests.

Anthropogenic gradient & stream name		Land use in the catchment			Hydrology				Ditching index	Stand age (years^{-1})	Habitat clearance index	TN (µg. L^{-1})	Turbidity (NTU)	Saturation in dissolved oxygen (%)
		% Agriculture land	% Clear-cuts	Distance to the nearest clear-cut (m)	V deviated (%)	Nb rises	Date min flow (Julian days)	Distance to the dam (m)						
Agriculture	Silverån	7.79	11.52	742	0.00	5	209.5	-	2.46	-	-	500.00	31.64	97.06
	Bulsjöån	17.65	11.01	1200	81.64	9	295	5300	2.39	–	–	574.00	5.34	93.31
	Borkhultsån	16.48	11.21	958	0.00	27	263	–	5.04	–	–	409.00	19.13	102.01
	Kisaån	7.44	14.11	280	0.00	23	302	–	2.73	–	–	432.00	3.54	91.50
	Pinnarpsbäcken	12.39	13.35	350	0.00	5	298	–	2.91	–	–	693.50	64.82	100.33
	Storån nedre delar	11.44	12.11	1306	84.88	20	302	1000	3.46	–	–	831.50	58.29	93.51
	Flemmabäcken	20.86	8.95	986	0.00	1	299	–	4.02	–	–	1840.00	144.23	88.85
	Börrumsbäcken	11.93	12.29	55	0.00	2.5	300.5	–	2.59	–	–	1805.50	447.87	88.93
	Kapellån	41.04	9.65	3570	0.00	27	263	–	2.65	–	–	2137.00	258.72	76.72
	Vadsbäcken	70.50	3.91	850	0.00	0.5	302	–	3.40	–	–	2225.50	611.99	77.12
River regulation	Hynnan	0.03	20.88	110	0.00	2.5	162	–	1.00	–	0.5	320.50	9.04	94.84
	Väjån	0.00	12.32	1720	0.00	2	164.5	–	1.48	–	0.4	329.00	2.96	86.83
	Götån	0.00	11.24	1276	0.00	5	164.5	–	1.18	–	0	293.50	72.44	97.50
	Likan	0.00	29.80	1240	0.00	3	164.5	–	0.97	–	0.1	243.50	18.05	97.00
	Acksjöälven	0.00	11.67	1740	58.5	14	227.5	135	1.84	–	0.7	338.00	4.62	93.87
	Lettan	0.16	17.75	610	205.6	67.5	216.5	740	1.42	–	0.2	293.00	10.45	96.45
	Hagälven	0.15	11.02	986	175.2	21.5	224	6400	2.59	–	0.5	272.00	0.93	97.47
	Halgån	0.00	15.15	529	93.8	29.5	183.5	380	1.14	–	0.9	234.50	0.24	94.03
	Örån	0.00	9.74	336	108.6	93	198	3300	0.99	–	1	215.50	46.74	92.12
	Tåsan	0.05	12.04	273	291.2	109.5	194	600	1.09	–	0.8	174.00	6.60	22.34
Forestry	Stortjärnbäcken	0.00	4.36	3700	0.00	2	58.5	–	2.42	87.10	–	367.50	92.19	92.97
	Fågelvinbäcken	2.55	0.41	993	5.50	12.5	170	–	0.70	69.97	–	592.50	606.22	89.90
	Stormyrbäcken	0.00	2.34	2800	0.00	3	58.5	–	2.13	74.90	–	375.50	25.55	88.18
	Kamplidenbäcken	0.00	3.60	830	0.00	1	147	–	2.02	70.94	–	471.00	162.06	89.43
	Hjuksvallbäcken	0.00	20.49	109	0.00	0.5	265.5	–	1.73	80.58	–	528.50	77.02	94.79
	Kläppmyrbäcken	0.00	3.61	916	0.00	1	147	–	2.62	66.74	–	487.00	272.31	83.99
	Brattmyrlidenbäcken	0.00	7.04	130	0.00	1.5	58.5	–	2.27	62.00	–	269.50	19.52	89.27
	Renbergsbäcken	0.00	13.23	149	0.00	1.5	58.5	–	1.86	74.88	–	304.00	83.59	93.77
	Svartbäcken	0.00	10.61	8	0.00	1.5	58.5	–	2.56	64.20	–	274.50	31.92	97.98
	Krycklan	0.00	12.27	92	0.00	1.5	58.5	–	1.36	63.40	–	269.00	8.85	94.13
	Bergmyrbäcken	0.00	5.89	152	0.00	3	58.5	–	2.66	60.30	–	267.00	6421.75	95.29
	Bastumyrbäcken	0.53	17.75	20	0.00	0	58	–	2.47	60.84	–	289.00	596.99	97.99
	Kvarnbäcken	0.07	16.70	105	0.00	0.5	58.5	–	4.37	63.28	–	621.50	111.86	96.06
	Västra Nybyggsbäcken	0.00	14.59	390	0.00	1	58.5	–	2.69	56.73	–	255.00	4.29	94.73
	Kluddbäcken	0.53	9.55	916	0.00	2.5	58.5	–	2.82	56.59	–	397.00	231.83	96.95
	Krickmyrbäcken	0.00	51.27	20	0.00	2	58.5	–	3.70	42.39	–	530.50	118.11	94.88

2.5. Ecosystem functioning assays

Algal biomass accrual was quantified on four pairs of unglazed tiles (25 × 25 cm, Seramiksan) in each stream reach over a period of 30 days in August. The pairs were anchored to the stream bottom, with edges of one tile of each pair coated with a layer of petroleum jelly to exclude invertebrate grazers (Lamberti and Resh, 1983). Algal biomass was measured using a BenthoTorch (bbe Moldaenke Gmbh, Kiel-Kronshagen, Germany), which converts measures of the fluorescence of chlorophyll *a* to an estimate of chlorophyll biomass (Kahlert and McKie, 2014), expressed as chlorophyll *a* mg m-2 day-1.

To assess litter decomposition, 5.0 ± 0.1 g of air-dried birch (*Betula* spp.) leaves were enclosed in mesh bags. The litter was drawn from a pooled sample, collected at abscission from sites outside of our sampling regions (59°48′42.1″N 17°39′47.1″E and 63°49′57.5″N 20°17′44.5″E), prior to the field seasons. Litterbags were either constructed from coarse mesh (10-mm mesh diameter), allowing colonization by both macroinvertebrates and microbes, or from fine mesh (0.5-mm) which excludes most macroinvertebrates and thus allows an estimate of the fraction of litter decomposition attributable to microbes. Five replicates of each mesh type were deployed in each stream for 42 ± 2 days, a period of time known to be sufficient for reaching 40–50% decomposition in Swedish reference sites (Frainer et al., 2014). After retrieval, leaves were cleaned under tap water, with invertebrates washed from the coarse bags retained and stored in 70% ethanol for later identification. Six 12-mm diameter leaf discs were cut from six different leaves in each bag and stored frozen for later assessment of fungal biomass. The remaining leaves were oven-dried for 48 h at 110 °C and then ashed at 550 °C for 4 h to quantify ash free dry mass (AFDM). Leaf mass loss was corrected for leaching of solutes, determined based on a laboratory trial. The breakdown rate coefficient *k* was calculated for each litterbag using the negative exponential decay model (Benfield, 1996).

Fungal biomass in the litter from the decomposition experiment was estimated based on the mass of ergosterol, a component of eumycotic cell walls (Gessner, 2005). Briefly, using alkaline methanol, ergosterol was extracted from freeze-dried leaf material and subsequently purified by solid-phase extraction (Sep-Pak® Vac RC tC18 500 mg sorbent; Waters, Milford, USA). Ergosterol concentration was quantified using high-performance liquid chromatography (HPLC; 1200 Series, Agilent Technologies, Santa Clara, USA) at a wavelength of 282 nm. Fungal biomass accrual was then standardized to $\mu g\ g^{-1}\ day^{-1}$, based on an assumption of negligible ergosterol accrual prior to the immersion in stream water (Krauss et al., 2005).

From the litterbags, detritivorous invertebrates denoted as leaf “shredders” (Cummins, 1974; Tachet et al., 2010) were counted and identified to the lowest taxonomic level possible.

2.6. Data analyses

All analyses mentioned below were conducted in R (R Core Team, 2015).

2.6.1. Anthropogenic gradients

Principle component analysis (PCA) on standardized variables was used to characterize and reduce the dimensionality of the anthropogenic gradients, with each gradient analyzed separately. Prior to the PCA, multicollinearity was assessed using Pearson correlation coefficient (PCC) and when two or more strongly correlated variables were present (PCC > 0.8), only one variable was kept – that which was least correlated with the remaining predictors. Based on the eigenvalues, the first two PCs were retained to characterize the dominant anthropogenic gradient in each region. The R package ade4 was used to run these analyses.

2.6.2. Community analysis

Canonical correspondence analysis (CCA) was used to correlatively assess the impact of environmental variables representing anthropogenic pressures on the community composition of the different organism groups, with the cca function from the R vegan package (Oksanen et al. 2013). Moreover, a Permutational Multivariate Analysis of Variance (PERMANOVA) was used to test whether there was a significant change in community composition across the anthropogenic gradients (PCs fitted as explanatory variables), run with the R function adonis, which can handle continuous predictors (Oksanen et al., 2015).

2.6.3. Regression analysis

Community and biodiversity metrics, i.e. total abundance (excepting diatoms as abundance cannot be estimated from valve count data), species richness, Shannon diversity, and Pielou evenness were computed and were checked for normal distribution of residuals and homoscedasticity. When needed, data were log-transformed. Linear models were used to determine whether these community metrics varied along the anthropogenic gradients, with fitting the eigenvectors of PC1 and 2 as explanatory variables as well as their interaction. Non-significant interactions were removed in order to get the most parsimonious models.

Similarly, we used linear mixed effect models (LMM) to assess anthropogenic effects on ecosystem process rates. For this, the eigenvectors of PC1 and 2 were fitted as our main predictor variables (fixed factor). An additional fixed factor was fitted to represent variables manipulated at the scale of the tile- or litterbag pair within streams: the presence/absence of petroleum jelly on the tiles or bag mesh size (coarse/fine) in the decomposition trial. These factors were tested against random factors that comprised stream identity (i.e. stream-specific variation in the responses) and litterbag or tile pair, nested within stream identity. The general model form was then: response ~ anthropogenic gradient * barrier + random(stream/replicate). All models were built with the R packages lme4 (Bates et al., 2015) and lmerTest for getting the associated p-values.

We chose to fit the two first eigenvectors of our PCA analyses rather than individual abiotic variables (e.g. nutrient concentrations or deviation in discharge) as predictors, since these were often correlated with other co-occurring stressors along the gradients. Use of the orthogonal PC axes as predictors avoids the risk of conferring misleading causality onto individual stressors, and emphasizes the anthropogenic gradients as covarying suites of stressors associated with broad classes of anthropogenic gradients. Nevertheless, the individual PC axes (PC1 and 2) often succeeded in orthogonally contrasting different impacts associated with each anthropogenic gradient.

To evaluate the relative strength of gradient effects on community responses, we first standardized our response variables and predictors (mean of zero and a standard deviation of one). This allowed calculation of scale-independent standardized partial regression coefficients (SPRC) along with their 95% confidence intervals (CIs), which we use to compare effect sizes (ES, expressed as unit SD) (Schielzeth, 2010). SPRC computations are slightly different when fitting LMM (Schielzeth, 2010): we first fitted a model with random intercepts to extract between-group SD. Second, we fitted a full model with the response standardized by its between-group SD. Therefore, the slope estimate from this model was qualitatively equivalent to SPRC calculated from group means. An ES expressed as percentage quantifies a relative change in a response variable between a treatment site (here, the most impacted site) compared to a control (here, the least impacted site).

Visualization along both dimensions of a gradient (PC1 and PC2) was achieved through two-dimensional surface plots displaying fitted response values from the GLM against a surface defined by the two PCs (PC1 on the X-axis and PC2 on the Y-axis; (Feld et al., 2016)). In addition to results $P < 0.05$, we also highlighted results $P < 0.1$, i.e. that explain variation in the data but where we lacked statistical power to detect any effects at the 5% level.

3. Results

3.1. Anthropogenic gradients

Agricultural gradient – The first two PCs captured 87% of the total variation (PC1: 65.4%). Percent agricultural land use within the catchment, turbidity, concentration of total nitrogen, and alkalinity increased along PC1, while dissolved oxygen saturation decreased (Table S1.2; Fig. S1.1a). PC2 was associated with increasing pH and catchment ditching (Table S1.2; Fig. S1.1a). PC1 and PC2 are hereafter denominated as the nutrient and agricultural ditching gradients, respectively.

River regulation gradient – The first two PCs explained 73.4% of the total variation (PC1: 39.7%). Deviation in water volume from the natural flow regime and number of times the magnitude of flow reversed (from high to low or vice-versa) increased along PC1, while concentrations of total nitrogen and TOC decreased (Table S1.2; Fig. S1.1b). These represent hydrological variables that fluctuate strongly in dams subjected to more frequent regulation, with water release from magazines on a weekly or daily basis, leading to more frequent occurrences of hydropeaking. PC2 represented a gradient ranging from mostly unregulated stream sites characterized by frequent low flow pulses, a faster rate of flow increase, an earlier date of minimum flow, lower water temperatures and fewer ditches in the catchment, to regulated stream sites characterized by more homogenous discharge (less low flow pulses and slower changes in flow), higher water temperatures and more ditches (Table S1.2; Fig. S1.1b). These characteristics are typical of longer term, seasonal regulation where water is released from magazines infrequently. Hereafter, PC1 is referred to as a hydropeaking gradient and, PC2 as a seasonal regulation gradient.

Forestry gradient – The first two PCs explained 65.8% of the total variation (PC1: 35.5%). PC1 captured variation in pH associated with increasing TOC and total P, and is hereafter called TOC gradient (Table S1.2; Fig. S1.1c). Variables characterizing forest management loaded predominantly onto PC2, which we hereafter term the forestry gradient. Percent of catchment clear-cuts and ditching were positively correlated with PC2 while percent of coniferous forest within the catchment and stand age characterized least-impacted stream sites (Table S1.2; Fig. S1.1c).

3.2. Responses of community composition and diversity along the anthropogenic gradients

Agricultural gradient: all organism groups respond to nutrient enrichment (H4) – Significant variation in the community composition of benthic diatoms, macroinvertebrates and fish was detected along the nutrient gradient, with macrophytes not significant at the 5% level (Table 2). In heavily impacted stream sites, communities shifted towards diatoms species such as *Eolimna subminuscula*, *Amphora pediculus*, *Planothidium frequentissimum* or *Surirella brebissonii* var. *kuetzingii* (Fig. S2.1a), invertebrate species including *Apatania* sp., *Hydraena* sp., *Asellus aquaticus* or *Gammarus pulex* (Fig. S2.2a) and fish such as sculpins (*Cottus gobio*) (Fig. S2.2b).

Significant variation in one biodiversity or abundance metric along the nutrient gradient (PC1) was detected for macrophytes, fish and diatoms, but not macroinvertebrates (Fig. 2a; Table S2.1), with diatom evenness (Fig. 3a) and macrophyte frequencies (Fig. 3b) increasing in heavily impacted sites (Effect size, ES: ~0.63–0.94;). As the nutrient gradient increased, fish diversity (Fig. 3c) and fish and macrophyte evenness generally decreased (~0.62; Fig. 2a), whereas benthic diatom diversity increased (0.45; Fig. 2a). In contrast, none of the biodiversity or abundance metric varied significantly along the ditching gradient (PC2; Table S2.1). However, stream sites with more ditching in their catchments showed generally decreased fish densities (~0.48; Fig. 2a) but increased fish evenness, diatom diversity and evenness (~0.35–0.49; Fig. 2a). The richness of all four organism groups responded to at least one of the nutrient and ditching gradients: from macrophyte richness nearly increasing by 0.40 along both dimensions of the gradient to fish richness decreasing by 0.51 along the nutrient gradient (Fig. 2a; Table S2.2).

River regulation gradient: fish and benthic invertebrates are the most impacted groups (H4) – Macrophyte community composition varied along the hydropeaking gradient (PC1, Table 2), with heavily impacted sites dominated by *Carex echinata*, *Juncus bulbosus* or *Sphagnum* (Fig. S2.1d).

Significant variation in biodiversity metrics along the hydropeaking gradient (PC1) was detected for diatoms and fish (Fig. 2b; Table S2.1), with increasing diatom richness and diversity, and fish evenness in heavily impacted sites (~0.66–0.68; $P < 0.05$; Fig. 3d-e-h). In contrast, fish densities (Fig. 3g) and richness decreased along this same gradient, and there was also a trend for reduced invertebrate diversity, (~0.30–0.63; Table S2.2), whereas diatom evenness increased (0.58; Fig. 3f). There were no statistically significant responses for any biodiversity or abundance metric for any of the studied organism groups along the seasonal regulation gradient (PC2; Fig. 2b; Table S2.1). However, there were some relatively large effect sizes (>0.2) in some organism group responses (Table S2.2). For example, macrophyte frequencies and richness and, invertebrate abundances increased with increasing impact along the seasonal regulation gradient (Fig. 2b; Table S2.2), whereas diatom evenness increased, invertebrate diversity and evenness were metrics decreasing as stream sites were more impacted by seasonal variation (Fig. 2b; Table S2.2).

Forestry gradient: diatoms and benthic invertebrates respond the most

Table 2
Outputs of the PERMANOVA testing for changes in community composition of the four organism groups, i.e. benthic diatoms, macrophytes, benthic invertebrates and fish, along anthropogenic gradients, i.e. agriculture (n = 10), river regulation (n = 10) and forestry (n = 16). Anthropogenic gradients are characterized by two dimensions that are extracted from separate principal component analyses (PC1 and PC2). For clarity, effects are only reported when the associated $P < 0.1$, with effects significant at the 5% level highlighted in bold. Corresponding ordination plots of these CCAs are available in supplementary material (Figs S2.1 & S2.2).

	Agriculture											
	Benthic diatoms			Macrophytes			Benthic invertebrates			Fish		
	F	R^2	P	F	R^2	P	F	R^2	P	F	R^2	P
Nutrients (PC1)	5.36	0.41	**0.005**	1.71	0.23	0.08	2.10	0.21	**0.05**	2.80	0.22	**0.005**
Agricultural ditching (PC2)	0.84	0.06	–	1.40	0.19	–	0.74	0.07	–	2.31	0.18	**0.005**
PC1*PC2	0.97	0.07	–	0.40	0.05	–	1.31	0.13	–	1.49	0.12	**–**
	River regulation											
Hydropeaking (PC1)	0.84	0.09	–	5.51	0.39	**0.01**	1.17	0.11	–	1.29	0.15	**–**
Seasonal regulation (PC2)	1.18	0.13	–	1.26	0.09	**–**	1.57	0.15	–	0.95	0.11	**–**
PC1*PC2	0.97	0.11	–	1.21	0.09	–	1.77	0.17	**0.05**	0.49	0.06	**–**
	Forestry											
Background TOC (PC1)	1.59	0.11	–	1.91	0.14	–	2.65	0.16	**0.02**	1.76	0.14	–
Forestry (PC2)	0.96	0.06	–	0.23	0.02	–	1.12	0.07	**–**	1.56	0.12	–
PC1*PC2	0.43	0.03	–	0.37	0.03	–	0.35	0.02	–	1.24	0.10	–

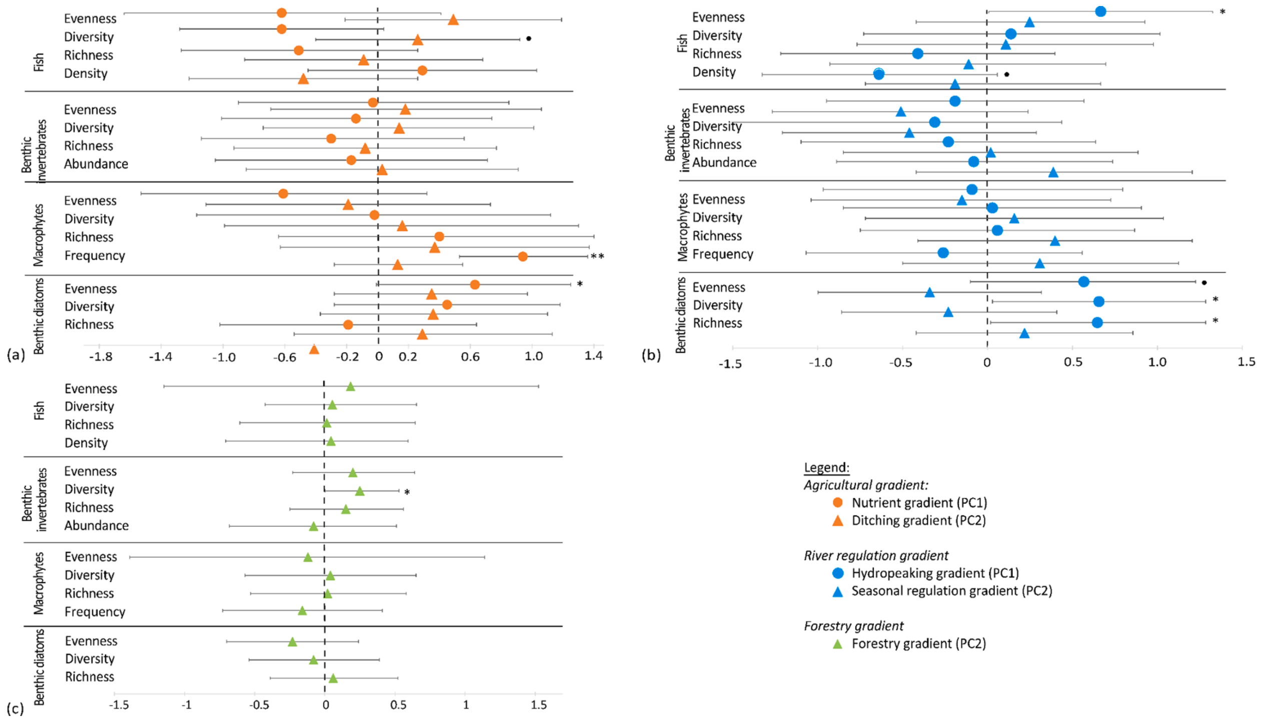

Fig. 2. Effect sizes (ES) and their confidence intervals (CI), calculated as standardized partial regression coefficients, of three anthropogenic gradients, i.e. agriculture (panel **a**, orange, n = 10), river regulation (panel **b**, blue, n = 10) and forestry (panel **c**, green, n = 16) on community composition indicators of four organism groups. Anthropogenic gradients were characterized as principal components (PC1 and PC2; one PCA analysis per gradient, respectively). No ES along the first dimension of the forestry gradient are presented as this PC represented background TOC variation between the streams rather than anthropogenic impacts. Significant effect sizes of dimensions of the anthropogenic gradients are indicated as following: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, • $P < 0.1$. Exact values of ES along with their 95% CI are available in Table S2.2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(H4) – Community composition did not change for any of the four organism groups along the forestry gradient (Table 2), but benthic invertebrate composition varied along the TOC gradient (PC1; Table 2; Fig. S2.1-2).

Significant variation in biodiversity metrics along the forestry gradient (PC2) was only observed for invertebrates (Table S2.1), with invertebrate diversity increasing along the pressure gradient (0.27; $P = 0.05$; Fig. 3i). While invertebrate evenness increased (0.20; Fig. 2c), diatom evenness decreased as the stream sites were more impacted by forestry (0.23; Fig. 2c).

3.3. Responses of ecosystem processes along the anthropogenic gradients

Agricultural gradient: a general increase in ecosystem processes (H1) – Neither algal biomass accrual, litter decomposition nor fungal biomass accrual varied significantly along the nutrient gradient (PC1; Fig. 4a; Table S3.2). However, there was a significant interaction for litter decomposition between bag mesh type and the nutrient gradient (Table S3.1), with decomposition in the coarse - but not fine mesh bags increasing along the nutrient gradient up to moderate levels of nutrient impact (Fig. S3.1). Fungal biomass accrual and shredder richness were negatively affected along the gradient (~0.40–0.43; Fig. 4a). Along the ditching gradient (PC2), fungal biomass accrual, shredder abundance and richness decreased while shredder evenness increased (Fig. 4a; Table S3.2).

River regulation gradient: algal biomass accrual and litter decomposition decline strongly (H2) Litter decomposition was the only ecosystem process rate that decreased significantly along the both dimensions of river regulation (Fig. 5c), i.e. hydropeaking and seasonal regulation (Fig. 4b), with an interaction between the two dimensions of the gradient being apparent (Fig. 5c; Table S3.1). Algal biomass accrual decreased along the hydropeaking gradient (PC1) by 0.33 but increased by 0.47 along the seasonal regulation gradient (Table S3.2). Fungal biomass accrual also increased greatly along the seasonal regulation gradient (PC2; Fig. 5b; Table S3.2). Shredder abundances increased along the hydropeaking gradient (0.32; Fig. 4b;) while their evenness decreased along both dimensions of the gradient (~0.36–0.43; Fig. 4b).

Forestry gradient: all ecosystem processes are suppressed (H3) – There was no effects of the forestry gradient on process rates for litter decomposition, algal biomass accrual or fungal biomass accrual (Fig. 4c; Table S3.2). Nevertheless, algal biomass and fungal biomass accrual increased as stream sites became more impacted by forestry (~0.36–0.38; Fig. 4c; Table S3.2).

4. Discussion

Our results provide insights into the particular combinations of structural and functional indicators that are most likely to give complementary information on the community and ecosystem impacts of three pervasive anthropogenic pressures: agricultural land use, river regulation and forestry (Table 3). Along the nutrient gradient, at least one aspect of community composition, abundance or biodiversity of each organism group responded. The strongest effect sizes (i.e. standardized partial regression coefficients) were observed for diatom evenness, fish diversity and evenness and, macrophyte abundance, while the greatest shift in community composition (with the largest r^2) along the gradient was observed for diatoms. Fish and diatoms were also useful for detecting additional impacts of ditching. Among the functional indicators, fungal biomass accrual showed the strongest effect size in response to both the nutrient and ditching gradients. The strongest responses to the hydropeaking gradient were observed for fish evenness, diatom richness and diversity, and in macrophyte community composition. Benthic diatoms and invertebrates were also good indicators of seasonal regulation. Among the functional indicators, litter decomposition responded to frequent hydropeaking, with large effect sizes also observed for algal biomass accrual in response to both hydropeaking and

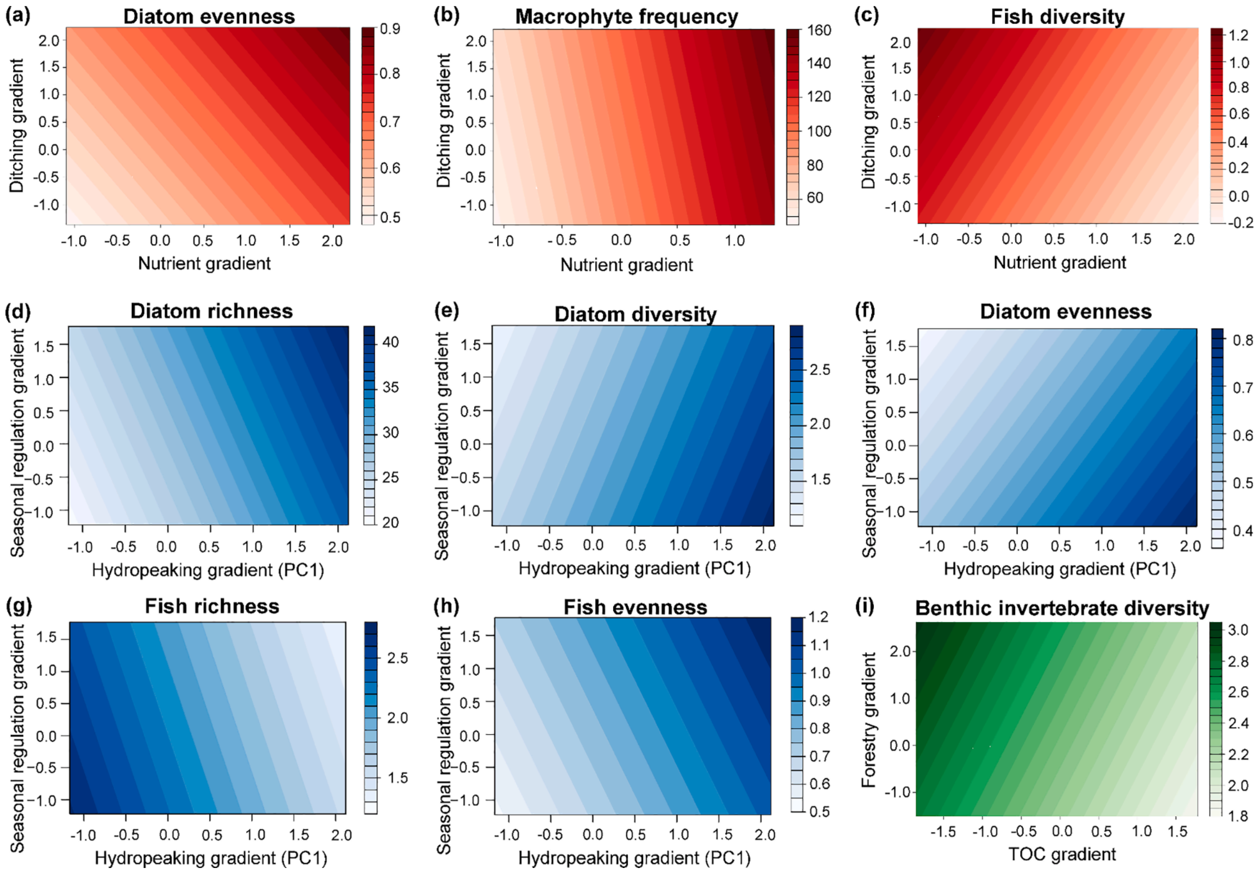

Fig. 3. Two-dimensional surface plots displaying the community metrics (abundance, richness, diversity and evenness) of four organism groups (diatoms, macrophytes, benthic invertebrates and fish) against a surface defined by the two main dimensions (i.e. PC1 and PC2) of the studied anthropogenic gradients, i.e. agricultural (red), river regulation (blue) and forestry (green) gradients. Graphs are plotted when at least one of the two relationships is significant at the 10% level. The greater the impact the higher the score along the anthropogenic gradient. Scatterplots for the same relationships are presented in Fig. S2.3-S2.5. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

seasonal regulation. Along the forestry gradient, invertebrate diversity was the only structural indicator responding, along with algal and fungal biomass accrual among the functional indicators. Overall, these results highlight the value of using complementary structural and functional indicators for detecting community and ecosystem level changes associated with different aspects of anthropogenic disturbance gradients.

The stressors associated with the nutrient gradient included not only increased nutrient concentrations, but also increased turbidity and reduced oxygen. Whereas nutrient enrichment often stimulates productivity and organic matter processing and can be associated with higher biodiversity (Woodward et al., 2012; Johnson and Hering, 2009), low dissolved oxygen and high levels of suspended mineral sediments have opposite effects, suppressing biological activity and biodiversity by blocking light, smothering habitats, abrading plant tissues and interfering with invertebrate feeding (Allan, 2004; Townsend et al., 2008). Overall, changes in structural metrics are indicative of a cumulative impact of this suite of stressors, with more tolerant species favored and diversity lowered as the agricultural impact increases (**H4**). This was confirmed as at least one structural aspect of each organism group varied along the nutrient gradient. For example, fish diversity decreased along the agricultural gradient (large effect size and nearly significant p-value), due to losses of species sensitive to changes in water chemistry, lower oxygen saturation, and fine sediments (Hering et al., 2006a; Kemp et al., 2011). Diatom communities shifted towards species that are tolerant to high nutrient concentrations or able to move in sediments (Hofmann et al., 2011), and benthic invertebrate communities were increasingly dominated by environmentally tolerant Diptera, Isopoda and Gastropoda in the more agricultural streams (Quinn, 2000; Quinn and Hickey, 1990; Tachet et al., 2010).

In contrast with the generally high response rate of structural indicators along the nutrient gradient, responses of the functional indicators were less consistent, refuting our initial hypothesis (**H1**). Indeed, only litter decomposition in the coarse bags was stimulated before reaching an asymptote when nutrient concentrations were moderate (e.g. Total P concentration = 166.5 μg L^{-1}), with no response detected for algal biomass accrual despite strong effects on diatom diversity and evenness. In their assessment of the response of litter decomposition across a continental-scale nutrient gradient, Woodward et al. (2012) argued that declines in litter decomposition at higher levels of nutrient enrichment were attributable to negative impacts of additional stressors associated with agricultural land use on the activity of decomposer organisms such as herbicides, fungicides and insecticides (Fernández et al., 2015; Schäfer et al., 2007), and it is possible that increasing pesticide use along our nutrient gradient contributed to the negative effect sizes observed for fungal biomass accrual. Additionally, the observed high levels of deposited and suspended mineral sediment in our more heavily impacted agricultural sites have potential to limit all measured ecosystem processes, by reducing light for algal growth, and smothering substrates and detrital material, abrading fungal biofilms (negative effect sizes) and limiting consumption by shredder (e.g. by inferring with mouthparts) (Piggott et al., 2012;, Sponseller and Benfield, 2001). Other explanations might also apply to the lack of responsiveness of the ecosystem processes to the nutrient gradient. For example, it is also possible that the asymptote observed in the response of litter decomposition to increasing nutrients is indicative that nutrients ceased to be limiting at that point in the gradient, while the lack of

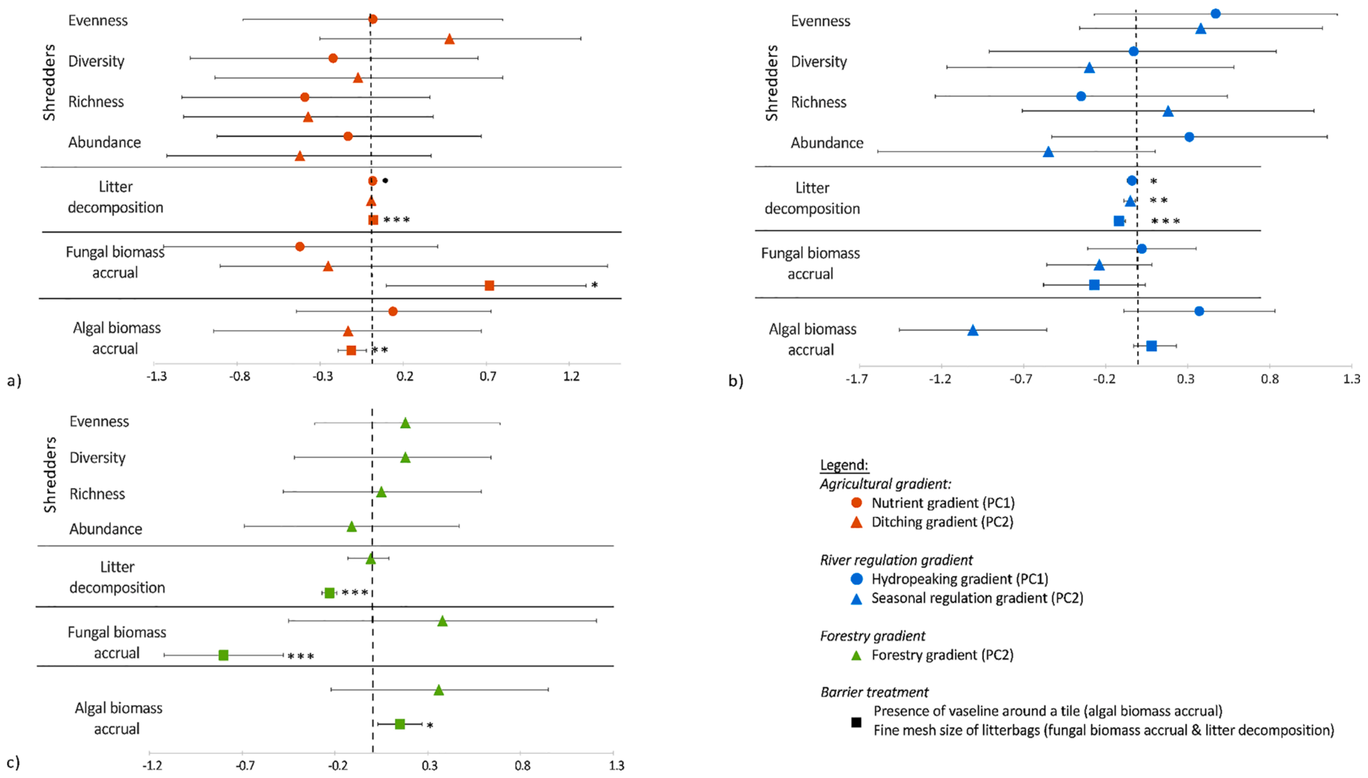

Fig. 4. Effect sizes (ES) and their confidence intervals (CI), calculated as standardized partial regression coefficients, of three anthropogenic gradients, i.e. agriculture (orange, n = 10), river regulation (blue, n = 10) and forestry (green, n = 16) on three ecosystem functioning indicators and shredder community metrics. Anthropogenic gradients were characterized as principal components (PC1 and PC2; one PCA analysis per gradient, respectively). No effect sizes along the first dimension of the forestry gradient are presented as this PC represented background TOC variation between the streams rather than anthropogenic impacts. Circles (PC1) and triangles (PC2) are pooling across barrier treatment while the squares show the effect sizes associated with the "barrier" treatment, pooling across gradients. Significant effects of dimensions of the anthropogenic gradients are indicated as following: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, • $P < 0.1$. Exact values of ES along with their 95% CI are available in Table S3.2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

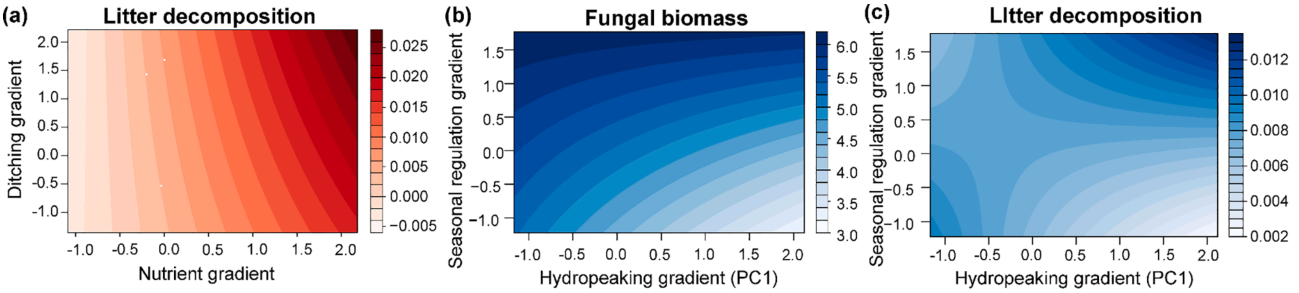

Fig. 5. Two-dimensional surface plots displaying ecosystem functioning indicators (algal biomass accrual and litter decomposition) and shredder abundance against a surface defined by the two main dimensions (i.e. PC1 and PC2 pooling across barrier treatment) of the studied anthropogenic gradients, i.e. agricultural (red) and river regulation (blue) gradients. For clarity only the significant responses are represented. The greater the impact the higher the score along the anthropogenic gradient. Scatterplots are also available in Figs S3.1–3.2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

variation in algal biomass accrual might reflect an increase in grazing pressure at high nutrient concentrations (Hladyz et al., 2011), . Further research is required to disentangle these potential explanations. Overall, the failure of a basal ecosystem process, as observed here especially for algal biomass accrual, to respond as expected along a strong underlying resource gradient, merits attention as potentially indicating functional impairment in a key food web compartment which is unlikely to be easily detected through structural based monitoring alone.

The river regulation gradient was defined not only by hydropeaking and seasonal water regulation but also by higher water temperatures and greater habitat simplification (i.e. lack of dead wood and vegetation in the stream channel) in the more impacted stream sites (Hedenskog et al., 2015; Ashraf et al., 2018). Effects of hydromorphological alteration have been most studied for fish and invertebrates (e.g. Pilloto et al., 2017; Göthe et al., 2019), and Hering et al. (2006a) suggested that these two groups are likely to be the best indicators for hydromorphological pressure in running waters. However, hydrological alteration has strong potential to affect water and habitat availability and hence key life history parameters (dispersal, reproduction) for all organism groups (Bragg et al., 2005; Riis and Biggs, 2003; Poff et al., 1997). We found that not only fish but also primary producers (i.e. macrophytes and diatoms) responded to the hydropeaking gradients

Table 3

Responses of fish (first row), benthic invertebrates (second row), macrophytes (third row), benthic diatoms (fourth row), functional processes (fifth row) and shredders (sixth row) to three anthropogenic gradients i.e. agriculture, river regulation and forestry on both structural and functional indices. Our working hypotheses were indicated as following: an increase in the response is in orange (either as an icon or as an arrow), a decrease in blue and the absence of response in black and, the larger the icon, the bigger the response of this organism group to the anthropogenic pressure. For each response, we reported our observed results using arrows: black arrows indicate a non-significant trend, orange arrows are for a significant increase while blue arrows indicate a significant decrease. The effects of a gradient on functional indicators are observed either as main effects (+) or in an interaction with the barrier treatment (*). Results with effect sizes > 0.2 only are reported in this table.

		Response	Agriculture gradient			River regulation gradient			Forestry gradient	
			Hypothesis	Nutrient	Agricultural ditching	Hypothesis	Hydropeaking	Seasonal regulation	Hypothesis	Forestry
Structural indices		Community composition		Yes	Yes					
		Density		↗	↘		↘			
		Richness		↘			↘			
		Diversity		↘	↗					
		Evenness		↘	↗		↗	↗		
		Community composition		Yes						
		Abundance						↗		
		Richness		↘			↘			
		Diversity					↘	↘		↗
		Evenness						↘		↗
		Community composition					Yes			
		Frequency		↗			↘	↗		
		Richness		↗	↗			↗		
		Diversity								
		Evenness		↘						
		Community composition		Yes						
		Richness			↗		↗	↗		
		Diversity		↗	↗		↗	↘		
		Evenness		↗	↗		↗	↘		↘
Functional indices		Litter decomposition	↗ (H1)	↗ *		↘ (H2)	↘ †	↗ †	↘ (H3)	
		Fungal biomass accrual	↗ (H1)	↘	↘			↗	↘ (H3)	↗
		Algal biomass accrual	↗ (H1)			↘ (H2)	↘	↗	↘ (H3)	↗
	Shredder	Community composition								
		Abundance			↘		↘			
		Richness		↘	↘					
		Diversity		↘						
		Evenness			↗		↘	↘		

(large significant effect sizes), while diatoms and invertebrates responded to the seasonal regulation gradient. Macrophyte communities shifted to a greater dominance to tolerant emergent/wetland species such as *Juncus bulbosus* and *Carex* sp. (Grime et al., 2007) in streams characterized by increased hydropeaking. Invertebrate diversity and evenness declined along the seasonal regulation gradient, with a similar large, but non-significant, effect size observed for diatom evenness, which might reflect the greater hydrological homogeneity of these sites compared with the references. Surprisingly, we observed several large positive effect sizes on diatom diversity metrics to the hydropeaking gradient. Frequent disruption of biofilm succession associated with more variable flows might result in greater heterogeneity in diatom communities successfully recolonizing individual stones (e.g. associated with founder effects), potentially favoring greater diversity at the reach scale (Biggs, 1995). Hydropeaking was associated with relatively large but non-significant (at the 5% level) negative effect sizes for richness and density, but increased fish evenness, suggesting that the smaller subset of the tolerant species known to characterize regulated rivers in Sweden (Göthe et al., 2019) also characterize our more impacted sites. Impacts of river regulation on stream communities remains far less investigated than impacts of nutrients and agriculture, and the frequency with which we observed large but non-significant positive and negative effect sizes in response to our regulation gradients highlights the need for further research on these impacts with a higher degree of replication than was possible here.

Studies evaluating relationships between hydrological variables and ecosystem processes often reported contradictory results (e.g. Ponsatí et al., 2015; Mbaka and Schäfer, 2015; Aristi et al., 2014). We observed a significant interaction between the two dimensions of our river regulation gradient on litter decomposition rates suggesting that these rates were lowest in streams with high hydropeaking but low seasonal regulation, and vice versa. This suggests that in some streams, a pattern of seasonal regulation helps to offset some of the negative impacts of hydropeaking on litter decomposition. However, more research is required to evaluate how general this result is, given the interaction was driven by responses of two sites in the middle of the hydropeaking gradient. Finally, despite the negative effects on diatom structural indices, we observed a large but non-significant effect size associated with the increase in algal accrual along the seasonal regulation gradient, refuting our hypothesis (**H2**) and, potentially reflecting the positive effects of higher temperatures and more stable discharges in the regulated relative to reference streams. This increase in algal biomass accrual could lead to priming effects, i.e. the production of labile organic carbon by algae that supports fungal biomass either directly or indirectly (bacterial necromass), as suggested by the positive effect size of fungal biomass accrual along the seasonal regulation gradient.

Forest clearcutting is known to have multiple impacts on the structure and functioning of adjacent stream channels (e.g. Stone and Wallace, 1998; Richardson and Béraud, 2014), many of which decline as riparian forest recovers (Lecerf and Richardson, 2010; McKie and Malmqvist, 2009). Overall, our results indicate that streams with an intact riparian zone are little affected by variation in forestry elsewhere in the catchment, refuting our initial hypotheses (**H3-4**). Indeed, only invertebrate diversity increased with forestry (significant p-value and relatively large size effect) and this increase did not translate to any of the functional responses. A larger number of metrics responded to variation in PC1, suggesting that, in the absence of direct effects of recent clearcutting, natural gradients in TOC and nutrients become more important than the extent of forestry in the catchment for explaining local biodiversity and ecosystem functioning (Petrin et al., 2007).

Palviainen et al. (2014) suggested a 30% threshold in the cover of clear-cuts necessary to see distinct effects of forestry on water chemistry in boreal streams,. Our results suggest a similarly high or higher threshold for impacts on community structure or ecosystem functioning, given only one of our sites was above this threshold (due to the loss of our two most heavily impacted streams which dried out prior to sampling). Still, the result observed for benthic invertebrate diversity and the large positive effect sizes for both fungal and algal biomass accruals – maybe due to the priming effects discussed above – suggest that forestry in the catchment may not be without consequences.

5. Conclusion

Our analyses demonstrate the potential for monitoring programs based solely on quantification of abiotic parameters and structural indicators (community composition, biodiversity) to miss important ecosystem-level impacts of human disturbances, including the strongly contrasting responses of different aspects of ecosystem functioning to anthropogenic gradients. The European Union's Water Framework Directive recommends incorporating measurements of ecosystem functioning into stream assessment routines (WFD, 2000), but does not clearly define what functional indicators should be considered. Gessner and Chauvet (2002) subsequently advocated for the use of litter decomposition rates as an indicator of stream functional integrity, since it is a process that integrates the activities of multiple organism groups over an extended period of time. However, the contrasting responses of our functional metrics to the anthropogenic gradients suggest that identifying a single and integrative functional indicator will be challenging. Larger scale approaches to quantifying ecosystem functioning, such as whole reach metabolism or nutrient uptake measurements, are possibly more integrative than the small scale measurements that were our focus, and thus might potentially yield more consistent responses along impact gradients. However, these larger scale measurements are conversely often more difficult to tie to specific changes in local assemblages or environmental characteristics.

Ultimately, the choice of biomonitoring approach should depend on management priorities, e.g. whether the focus is on biodiversity conservation (i.e. monitoring community structure) or on final ecosystem services (i.e. monitoring ecosystem processes). Our results provide insights into the particular combinations of structural and functional metrics that are most likely to give complementary information on the community and ecosystem impacts of different types of anthropogenic pressures. In particular, the effects of agriculture and river regulation were most strongly reflected by diatom and fish communities and changes in litter decomposition. However, monitoring of ecosystem processes also requires clear benchmarks (Frainer et al., 2021), to assist in identifying which levels of functioning should be regarded as degraded. Such benchmarks should further account for the possibility that a lack of response in a functional metric might also be indicative of functional impairment, as possibly seen in the lack of response of algal biomass accrual along our nutrient gradient. If the lack of response of algal biomass accrual to the increase in nutrient concentrations along the agricultural gradient is due to a co-occurring stressor, such as elevated sediments or pesticides, it might be indicative of lower retentivity by epilithic biofilms, and a profound alteration in ecosystem functioning. Increases in algal productivity with increasing nutrients is one mechanism by which excess nutrients are retained at local-stream reach scales, leading to tighter nutrient spirals and reduced rates of nutrient export to downstream lakes, estuaries and oceans (Mulholland et al., 2008, Newbold et al., 1981).

6. Authors' contributions

AT, FE, DGA, MK, RKJ and BGM designed the study. AT, MK, MB collected the data. AT analyzed the data. AT, RS and BGM led the writing of the manuscript and all other authors commented on earlier drafts.

7. Data availability

Data that supports the findings of this study will be available at the Swedish National Data Service.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.108514.

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