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Ecological stoichiometry of indirect grazer effects on periphyton nutrient content

Helmut Hillebrand · Paul Frost · Antonia Liess

Abstract Ecological stoichiometry has been successful in enhancing our understanding of trophic interactions between consumer and prey species. Consumer and prey dynamics have been shown to depend on the nutrient composition of the prey relative to the nutrient demand of the consumer. Since most experiments on this topic used a single consumer species, little is known about the validity of stoichiometric constraints on trophic interactions across consumers and ecosystems. We conducted a quantitative meta-analysis on grazer–periphyton experiments to test (1) if benthic grazers have consistent effects on the nutrient composition of their prey, and (2) whether these effects can be aligned to the nutrient stoichiometry of grazer and periphyton, other environmental factors, or experimental constraints. Grazers significantly lowered periphyton C:N and C:P ratios, indicating higher N- and P-content of grazed periphyton across studies. Grazer presence on average increased periphyton N:P ratios, but across studies the effect size did not differ significantly from zero. The sign and strength of grazer effects on periphyton nutrient ratios was strongly dependent on the nutrient content of grazers and their food, but also on grazer biomass, the amount of biomass removal and water column nutrients. Grazer with low P-content tended to reduce periphyton P-content, whereas grazers with high P-content increased periphyton P-content. This result suggests that low grazer P-content can be an indication of physiological P-limitation rather than a result of having relatively low and fixed P-requirements. At the across-system scale of this meta-analysis, predictions from stoichiometric theory are corroborated, but the plasticity of the consumer nutrient composition has to be acknowledged.

Keywords Meta-analysis · Homeostasis · Plasticity · Indirect interactions · Plant–herbivore interactions

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Introduction

The understanding of trophic interactions has been fostered by acknowledging the importance of material imbalances between consumers and their prey as summarized in considerations of ecological stoichiometry (ES). The appeal of ES lies in the fact that it provides a general framework spanning different levels of ecological organization linking cellular processes, organism physiology, population growth and the outcome of trophic interactions (Sterner and Elser 2002; Elser et al. 2003). ES has been successfully applied to population ecology to understand resource allocation, life history plasticity (Jeyasingh and Weider 2005) and

population growth (Ågren 2004; Vrede et al. 2004). It has played a special role in community ecology in order to explain consumer responses to prey quality (food intake, growth) (Cross et al. 2003; Fagan and Denno 2004; DeMott and Pape 2005), competition between consumer species (Hall 2004; Loladze et al. 2004) and consumer effects on prey nutrient composition (Daufresne and Loreau 2001; Hessen et al. 2002; Urabe et al. 2002). In addition, ES has provided new and comprehensive explanations for whole-ecosystem patterns in nutrient fluxes and food web structure (Elser 2000; Elser et al. 2000b; Darchambeau et al. 2005).

A central focus of ES is the mismatch between the consumer's demand for essential nutrients and the availability of these nutrients in their prey (Sterner and Elser 2002). Therefore, the interaction between plants and their herbivores plays a pivotal role in ES because autotrophs show a strong variability in nutrient content (Hillebrand and Sommer 1999; Quigg et al. 2003; Klausmeier et al. 2004), whereas herbivorous animals typically show a more confined stoichiometry (Andersen and Hessen 1991; DeMott and Pape 2005). This is largely due to autotrophs possessing (and consumers not) the ability to change internal cellular pools of nutrient-rich biomolecules and to store excess nutrients (Frost et al. 2005a). Given their relatively constant elemental composition, herbivores growing at maximal rate require a particular ratio of elements in their food. Such relative nutrient demands create the possibility that not only carbon (C) but other nutrients (most importantly phosphorus, P, and nitrogen, N) can be growth limiting for the consumer (Boersma and Kreutzer 2002; Frost et al. 2006).

Much of the basis for our current understanding of ES has been derived from field and experimental studies in pelagic aquatic systems (Hassett et al. 1997; DeMott and Gulati 1999; Elser et al. 2000b; Paterson et al. 2002). However, ES has also proved useful for studies in terrestrial (Fagan and Denno 2004; Kay et al. 2004) and in aquatic benthic ecosystems, especially involving periphyton and their invertebrate grazers (Hillebrand and Kahlert 2001; Frost and Elser 2002b; Frost et al. 2002b; Stelzer and Lamberti 2002). In the benthos, observational studies found considerable between-taxa variability of grazer elemental composition and a strong mismatch to the stoichiometry of their food (Frost et al. 2003; Evans-White et al. 2005; Liess and Hillebrand 2005; Fink et al. 2006). Experimental studies have revealed a positive relationship between grazer presence and the N and P content of periphyton (Hillebrand and Kahlert 2001; Frost et al. 2002b; Evans-White and Lamberti 2006), a dependence of algal nutrient content on nutrient supply (Hillebrand and Sommer 1999; Stelzer and Lamberti 2001; Frost and Elser 2002a) and a positive effect of low C:N and C:P ratios in the algae on grazer growth rates (Frost and Elser 2002b; Stelzer and Lamberti 2002).

Recently, Hillebrand et al. (2004) showed that light supply affected the stoichiometry of grazer–periphyton interactions and that N-limited interactions yielded results which were in line with expectations derived from previous P-limited experiments.

Although ES has been used to study benthic systems, interactions between grazers and periphyton in these systems are complicated by a number of system-specific characteristics. A major discrepancy between pelagic and benthic herbivore–plant interactions is based on the multitude of pathways by which benthic grazers can affect the stoichiometry of periphyton (Frost et al. 2002b) (Fig. 1). First, periphyton is a complex community that includes a number of different organisms and a considerable amount of dead organic matter, detritus. Empirical evidence suggests that non-algal material can be a dominant part of the organic material in periphyton (Frost et al. 2005b). The proportion of detritus is reduced by the unselective grazing of many benthic invertebrates, which remove both algae and detritus, but only the algae regenerate. Therefore grazing increases the proportion of live organisms compared to detritus. By reducing the proportion of C-rich but nutrient-poor detritus, grazers may increase the relative nutrient content of the remaining periphyton. Second, nutrient uptake of intact periphyton mats is often slower than cell-specific uptake rates as boundary effects reduce the uptake
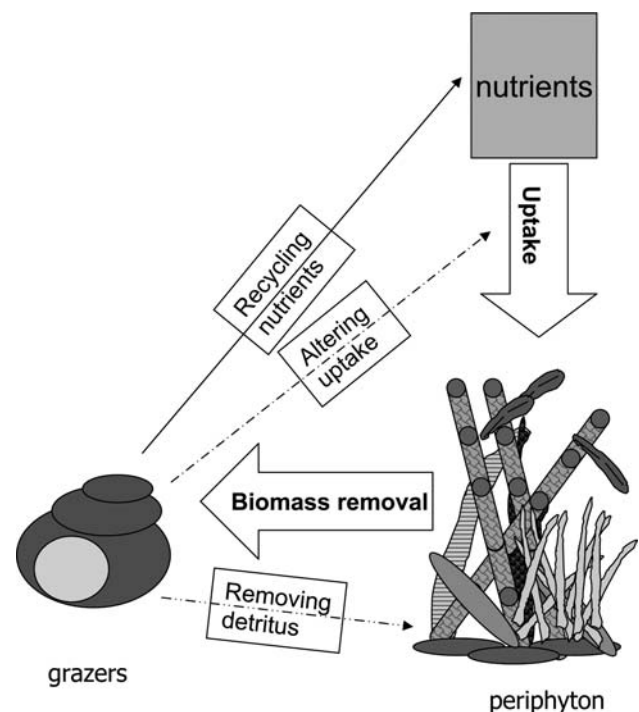
Fig. 1 Illustration of direct and indirect effects of grazers on nutrient stoichiometry. Three different pathways of how grazers can affect periphyton C:N:P ratios are exemplified: altered nutrient uptake, removal of detritus and nutrient recycling (see "Introduction" for details)

ability of the benthic algae (Riber and Wetzel 1987; Bothwell 1989; Burkholder et al. 1990). By altering the architecture of the periphyton community and by increasing the relative availability of nutrients per unit biomass remaining, grazers may enhance periphytic nutrient uptake and as a consequence increase its nutrient content. Nutrient uptake rates ultimately also change periphyton stoichiometry if grazers alter species composition or simply enhance biomass turnover. Third, the excretion and/or egestion of nutrients by grazers also increase the supply of nutrients available to the periphyton. In this case, grazers may spatially recycle nutrients (i.e., within the boundary layer) that increases the availability and uptake of nutrients by the periphyton. In streams, however, grazers may increase the export of nutrients (Mulholland et al. 1991). All three pathways may work at the same time and they are difficult to disentangle experimentally.

In spite of the considerable progress made in explaining the stoichiometric interplay between nutrient ratios, autotrophs and herbivores, the main body of evidence still consists of experiments with a limited number of consumer species—mostly from pelagic studies using the cladoceran genus *Daphnia* (Sterner and Hessen 1994; DeMott and Gulati 1999; Sommer et al. 2003). It thus remains unclear whether grazer effects on prey biomass and nutrient content (C:N and C:P ratios)—as it relates to their nutrient stoichiometry—varies among species and across study systems. We lack information about how grazers differing in their N- and P-content alter the outcome of consumer–prey interactions, especially in light of contrasting expectations from constrained (relative homeostasis) versus plastic (flexible) nutrient composition of the consumer.

Therefore, we analyzed the effects of grazers on periphyton nutrient content across ecosystems and consumer groups. We focus on grazer–periphyton interactions here as we were able to assemble a dataset comprising >100 grazer exclusion experiments where effect sizes on periphyton stoichiometry were available and a number of additional variables potentially explaining these effects were recorded. We examined benthic grazer effects on periphyton stoichiometry and tested whether these effects were consistent across lotic, lentic and coastal ecosystems or could be aligned to stoichiometric constraints, environmental variables or experimental details (see details below). Specifically, we asked,

1. Whether grazer effects on periphyton C:P, C:N or N:P ratios differed between ecosystems. We expected such differences as nutrient uptake and regeneration differs between lotic and lentic habitats because of the unidirectional flow in streams and between coastal and freshwater habitats because of different nutrient limitations.

2. Whether these effects depended on the stoichiometry of resources within the grazers, the periphyton and dissolved nutrient pools.
3. Whether these effects depended on the amount of periphyton biomass removed by the grazer or the composition and biomass of the grazer community.
4. Whether these effects depended on abiotic constraints of the environment (temperature, light) or experimental duration.

Methods

The data were derived from a database containing >800 experiments manipulating grazer presence and analyzing periphyton biomass in replicated treatments (Hillebrand 2008). This database is continuously updated by searching literature databases such as Web of Science and Aquatic Science and Fisheries Abstracts. For this analysis, we only used grazer exclusion studies reporting the effect of grazer presence on periphyton nutrient stoichiometry (C:N, C:P or N:P ratios). This corresponded to a total of 20 studies, which contained the results of 119 experiments [Table S1 in Electronic supplementary material (ESM)].

We used log response ratios (LR) to measure the effect of grazer presence on periphyton C:N, C:P or N:P ratios (LR-CN, LR-CP and LR-NP) and on algal biomass (LR-B), respectively. LR have been successfully used to quantify effect sizes in meta-analysis (Hedges et al. 1999; Rosenberg et al. 2000) and are—as we deal with changes in ratios—preferred in this case since they measure the proportional change in the response variable due to the grazer manipulation. For all LR, the effect size is the natural log-transformed ratio of the mean value at grazer presence to the mean value at grazer absence (Hedges et al. 1999; Rosenberg et al. 2000). Negative LR-B thus indicates a decrease in algal biomass with grazer presence. Negative LR-NP indicates a decrease in periphyton N:P ratios with grazer presence, which represents a relative increase of P over N.

In order to test what controls grazer effects on periphyton nutrient ratios, we also obtained the following information for each of the experiments (when presented, see ESM S1). In categorical variables, we grouped the habitat (lotic, lentic, coastal) and the consumer group (gastropods, arthropods, and diverse, the latter comprising studies with other grazers and those with natural grazer assemblages). In continuous variables, we recorded the duration of the experiment (log days), the molar ratio of dissolved inorganic nitrogen (DIN) to soluble reactive phosphate (SRP) in the water column (log N:P), the ratio of mean irradiance (μmol photons m^{-2} s^{-1}) to DIN (log L:N) and irradiance to SRP (log L:P), the average water temperature (°C), the

grazer biomass (mg dry weight m^{-2}) and body size (g wet weight), the C:N, C:P and N:P ratios of the grazers. We only used studies where grazer C:N:P was measured directly for the organisms involved in the experiment. In addition, we measured the mismatch between consumer nutrient demand and periphyton nutrient content by using the ratio of grazer C:N, C:P or N:P to periphyton C:N, C:P or N:P, respectively. For example, a mismatch >1 for grazer C:P to periphyton C:P means that grazers have higher relative C-content than their food. A mismatch <1 for grazer N:P to periphyton N:P means that grazers have lower relative N-content than their food, but higher relative P-content. With regard to mismatch variables, please note that—within the same analysis—we did not use the same nutrient ratio in the mismatch as in the effect size as to avoid spurious correlations. For example, for the analysis of LR-NP we used grazer—periphyton mismatches for C:P and C:N, but not for N:P. Additional potentially interesting predictor variables such as TN:TP ratios or predator presence could not be obtained in a consistent way from the primary studies.

We used an hierarchical approach to the analysis of these data. First, we calculated overall average effects sizes and their 95% confidence intervals (CI) across the entire data set, conducting a weighted meta-analysis (Rosenberg et al. 2000). The weighting is based on a measure of sampling variance for the effect size (Hedges et al. 1999; Rosenberg et al. 2000). For LR, the sampling variance is calculated from the number of replicates and the standard deviation from the mean values at grazer presence and absence (Rosenberg et al. 2000). An overall effect size is considered significantly positive or negative if the 95% CI does not include zero.

Second, we tested for univariate effects of the explanatory variables on the effect sizes (LR-CN, LR-CP and LR-NP). We used categorical weighted mixed-model analyses to test for significant differences between lotic, lentic and coastal ecosystems and between gastropod, arthropod or other grazers. For the other variables, we used a continuous weighted mixed-model meta-analysis, which is comparable to a weighted regression, delivering parameters of a linear regression in addition to a test of significance. All mixed-model analyses were performed with MetaWin 2.0 (Rosenberg et al. 2000). We employed Bonferroni-adjustments of significance values to account for the multiple tests performed with each dependent variable. As Bonferroni adjustments are considered highly conservative (Quinn and Keough 2002), we report significant effects when $P < 0.0067$ (corresponding to P adjusted <0.1). Please note that the number of studies (k) involved in the different statistical tests differs as not all measures were reported for all experiments (see "Results").

The advantage of these meta-analyses is that they consider the sampling variance of the single studies by weighting. The disadvantage however, is that these separated analyses do not account for covariance between explanatory variables. Therefore, as a final approach, we used a model selection procedure employing the Akaike information criterion (AIC) to test for significant relationship between effect sizes and explanatory variables in light of the other variables (Johnson and Omland 2004). This resulted in a most parsimonious model explaining the largest variance with a minimum number of variables. However, this approach required that a study provided information on all relevant predictor variables, reducing the data set considerably. In order to balance the loss of studies and the number of variables included in the model selection, we also reduced the set of predictor variables. Light:P ratio, light:N ratio, water temperature and grazer body size were not considered, as these variables were often not reported in the original studies and had limited effects in the univariate regressions.

Thus, we performed two statistical analyses in order to compare a univariate weighted approach on a large dataset (but without accounting for covariance between predictor variables) with a multivariate general linear model which accounted for covariance in a smaller dataset (but without weighting). For the remainder of the text, we use MA to abbreviate the weighted meta-analysis and GLM to abbreviate the most parsimonious multivariate model. We provide full statistical results as an online appendix (ESM S2), but summarize the statistics in the result section.

Results

Average effect sizes

The effects of grazer presence on periphyton C:N and C:P varied strongly among experiments (Fig. 2). LR-CN ranged from −1.03 to 0.68 (corresponding to a range from 64% reduction to 96% increase in C:N). The range was −0.83 to 1.29 (−56 to +262%) for LR-CP and −0.82 to 1.12 (−56 to +205%) for LR-NP. Despite this variation, significant negative overall effect sizes were found across all experiments for both LR-CP and LR-CN: grazer presence on average decreased periphyton C:N (average LR-CN = −0.088; 95% CI: −0.124 to −0.054) and C:P ratios (average LR-CP = −0.075; 95% CI: −0.135 to −0.011) (Fig. 2). The average effect of gazer presence on periphyton N:P was positive (0.051), but marginally not significantly different from zero (95% CI: −0.007 to 0.109) (Fig. 2).

The effect sizes on periphyton stoichiometry were strongly correlated to stoichiometric and environmental
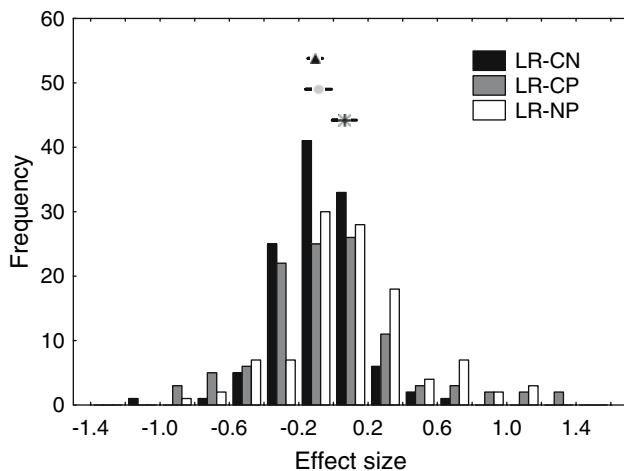
Fig. 2 Frequency distribution of grazer effect sizes on periphyton C:N, C:P and N:P, respectively. At the *top* of the diagram, average effect sizes and their 95% confidence intervals are given

constraints (see below). In the following, we focus on factors with significant influence in both univariate and multivariate analyses.

Effects on periphyton C:N (LR-CN)

Both MA and GLM indicated that LR-CN correlated positively with grazer body C:P and LR-B (Table 1). Grazer presence led to lower C:N (higher periphytic N-content) when grazer contained much P (low C:P) (Fig. 3a) and when they removed large portions of the biomass (negative LR-B) (Fig. 3c). The final GLM also included the ratio of available N:P, which was not significant in the MA (Table 1). At low N:P ratios (low relative availability of N), grazers had a stronger negative effect on periphyton C:N (Fig. 3b).

In the MA, more significant effects were found which disappeared in the GLM. There are two potential mechanisms for this divergence: First, accounting for the covariance with other factors, such factors may actually disappear or reverse in the GLM. Second, the trend might only be found in the larger data set used for the MA and disappear because studies were missing in the smaller data set for the GLM. We present these effects here, but they should be interpreted cautiously.

Table 1 Summary of results of univariate weighted meta-analysis (MA) and multivariate analysis (GLM) of grazer effects on periphyton C:N (LR-CN), C:P (LR-CP) and N:P (LR-NP)

Factor	NPLRR				CPLRR				CNLRR			
	MA		GLM		MA		GLM		MA		GLM	
	P	Slope	P	Slope	P	Slope	P	Slope	P	Slope	P	Slope
Habitat	0.7143		NI		0.8921		NI		**0.0000**		NI	
Consumer group	*0.0150*		**0.0048**		**0.0014**		**0.0087**		0.1763		NI	
Available N:P	*0.0470*	0.153	NI		**0.0063**	0.190	**0.0000**	1.045	0.0588	0.076	**0.0002**	0.266
Available light:P	*0.0367*	−0.062	–	–	**0.0048**	−0.088	–	–	0.4001		–	–
Available light:N	*0.0217*	−0.069	–	–	*0.0292*	−0.067	–	–	0.2786	0.017	–	–
Temperature	**0.0015**	0.024	–	–	0.2362	0.009	–	–	0.8065	0.001	–	–
Grazer biomass	*0.0432*	0.175	**0.0000**	3.320	**0.0000**	0.360	**0.0000**	2.756	0.8813	−0.007	NI	
Grazer body size	*0.0134*	0.162	–	–	0.2911	0.065	–	–	**0.0064**	−0.081	–	–
Grazer CP	0.3344	0.001	0.1517	0.002	*0.0136*	0.003	**0.0201**	0.023	**0.0000**	0.002	**0.0187**	0.001
Grazer CN	0.7443	0.040	NI		0.4118	−0.112	0.0565	−0.688	*0.0075*	−0.184	NI	
Grazer NP	0.1493	0.004	NI		**0.0010**	0.009	**0.0214**	−0.110	**0.0001**	0.008	NI	
Exp. duration	0.1430	−0.167	NI		0.0880	−0.206	NI		**0.0000**	−0.336	NI	
LR-B	0.4668	0.030	0.1385	−0.134	**0.0000**	0.154	NI		**0.0000**	0.096	**0.0020**	0.122
Mismatch NP	–	–	–	–	**0.0000**	0.038	NI		0.1465	0.010	NI	
Mismatch CP	*0.0439*	0.048	**0.0023**	−0.218	–	–	–	–	**0.0000**	0.071	NI	
Mismatch CN	0.3919	0.331	NI		**0.0003**	1.402	**0.0004**	−2.666	–	–	–	–
Model F (df)			13.32 (5,23)				23.24 (7,21)				31.16 (3,25)	
Model P			0.0000				0.0000				0.0000	
Model R^2			0.6874				0.8476				0.7636	

The table lists the factors (see "Methods" for details), for each univariate analysis the significance value (*P*) and the slope of the weighted regression, and for the GLM the slope for each term included in the final model and the analysis of variance for the entire model (including the *F*-ratio, the overall significance level, and the explained variance *R²*). *Bold* significance levels for the univariate tests indicate significance after Bonferroni-adjustment (adjusted $P < 0.1$). Complete information on all significance tests can be found in ESM S2

Italic significance levels are for univariate tests which were significant at $P < 0.05$ before Bonferroni-adjustment
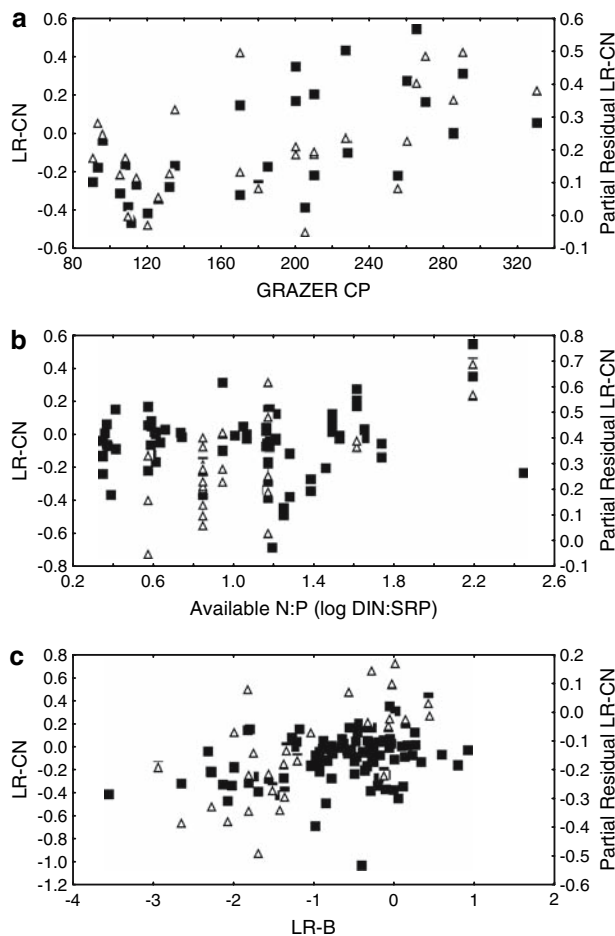
NI not included in the final GLM, – not tested

Fig. 3 Bivariate plot of grazer effect sizes on periphyton C:N (LR-CN) across gradients of **a** grazer C:P ratio **b** ratio of dissolved N:P, and **c** grazer effects on periphyton biomass. Solid symbols (*filled square*) are raw effect sizes from the single experiments (*left axis*); open symbols (*open triangle*) are partial residuals of LR-CN for that factor (i.e., removing the effects of other factors in the multivariate analysis) (*right axis*)

Only in the MA, LR-CN was positively correlated to the grazer–prey mismatch in C:P and the grazer N:P, but negatively correlated to the duration of the experiment and grazer body size (Table 1). Thus, positive effects of grazers on periphyton N-content (negative LR-CN) were restricted to grazers having low relative N content (low grazer N:P) and less carbon than their prey (mismatch CP ≤1) as well as to large grazers in long-lasting experiments. None of these variables were included in the final GLM, neither was the difference between habitats, where negative LR-CN were strongest in lotic habitats (average LR-CN = −0.209), still significantly negative in coastal habitats (average LR-CN = −0.112), but weak in lentic habitats (average LR-CN = 0.005).

Effects on periphyton C:P (LR-CP)

Both MA and GLM converged upon a significant difference in LR-CP between consumer groups and a positive correlation

between LR-CP and the ratio of available N:P, grazer biomass, and grazer C:P, respectively (Table 1). A diverse grazer assemblage reduced periphyton C:P more than gastropods, and even more negative LR-CP were found for arthropod grazers (Fig. 4a). Negative LR-CP were also confined to experiments with low grazer biomass (Fig. 4b), to grazers with low C:P ratios (Fig. 4d), and to situations with high P-availability (low N:P ratios; Fig. 4f). The GLM also included a marginally non-significant effect of grazer C:N, which suggested strongest reduction in C:P with highest grazer C:N (Fig. 4c). This effect had the same tendency, but was not significant in the MA (Table 1).

In both statistical analyses, LR-CP was additionally affected by grazer N:P and by the mismatch of grazer to periphyton C:N, but the analyses showed opposite directions. With increasing grazer N:P, the raw effect sizes increased, but the residuals from the GLM decreased (Fig. 4e). The reason is probably that LR-CP correlated in a similar way to grazer C:P and grazer N:P, and only the former trend was conserved in the GLM. Also the effect of C:N mismatch varied between the analysis, with increasing mismatch leading to a reduction in periphyton C:P in the raw data, whereas the GLM residuals showed the opposite trend (Fig. 4g).

The weighted MA also detected significant effects of LR-B, the mismatch in N:P and the available light:P ratios (Table 1). Negative LR-CP were related to efficient biomass removal (LR-B) and grazers having little deviation in their NP compared to their prey. Thus, grazers which contained relatively less P than the periphyton tended to increase periphyton C:P. Lower relative availability of P (higher light:P ratios) led to more negative LR-CP effect sizes.

Effects on periphyton N:P (LR-NP)

In the univariate analyses of LR-NP, only the temperature effect remained significant after Bonferroni-adjustment (Table 1), with positive effects on periphyton N:P related to higher temperatures. The GLM included five factors, three of which showed the same trends in the MA, which became non-significant due to the adjustment (Table 1). In the GLM, diverse grazer assemblages had negative effects and gastropod grazers had positive effects on periphyton N:P (Fig. 5a). Effects on periphyton N:P were also negative when grazer assemblages had little biomass (Fig. 5b) and small effects on algal biomass (Fig. 5d). High grazer C:P (low P-content) resulted in positive LR-NP (Fig. 5c), thus grazers containing little P enhanced N over P-content for their food. Increasing mismatch between grazer C:P and periphyton C:P also resulted into more negative LR-NP in the GLM (Fig. 5e), as grazers with higher C than their prey enhance the relative P-content.

Fig. 4 Bivariate plot of grazer effect sizes on periphyton C:P (LR-CP) for different consumer groups (**a**) (*Gas* gastropods, *Div* diverse, *Art* arthropods) and across gradients of **b** grazer biomass, **c** grazer C:N ratio, **d** grazer C:P ratio, **e** grazer C:P ratio, **f** ratio of dissolved N:P, and **g** C:N mismatch between grazer and periphyton. *Symbols* as in Fig. 3
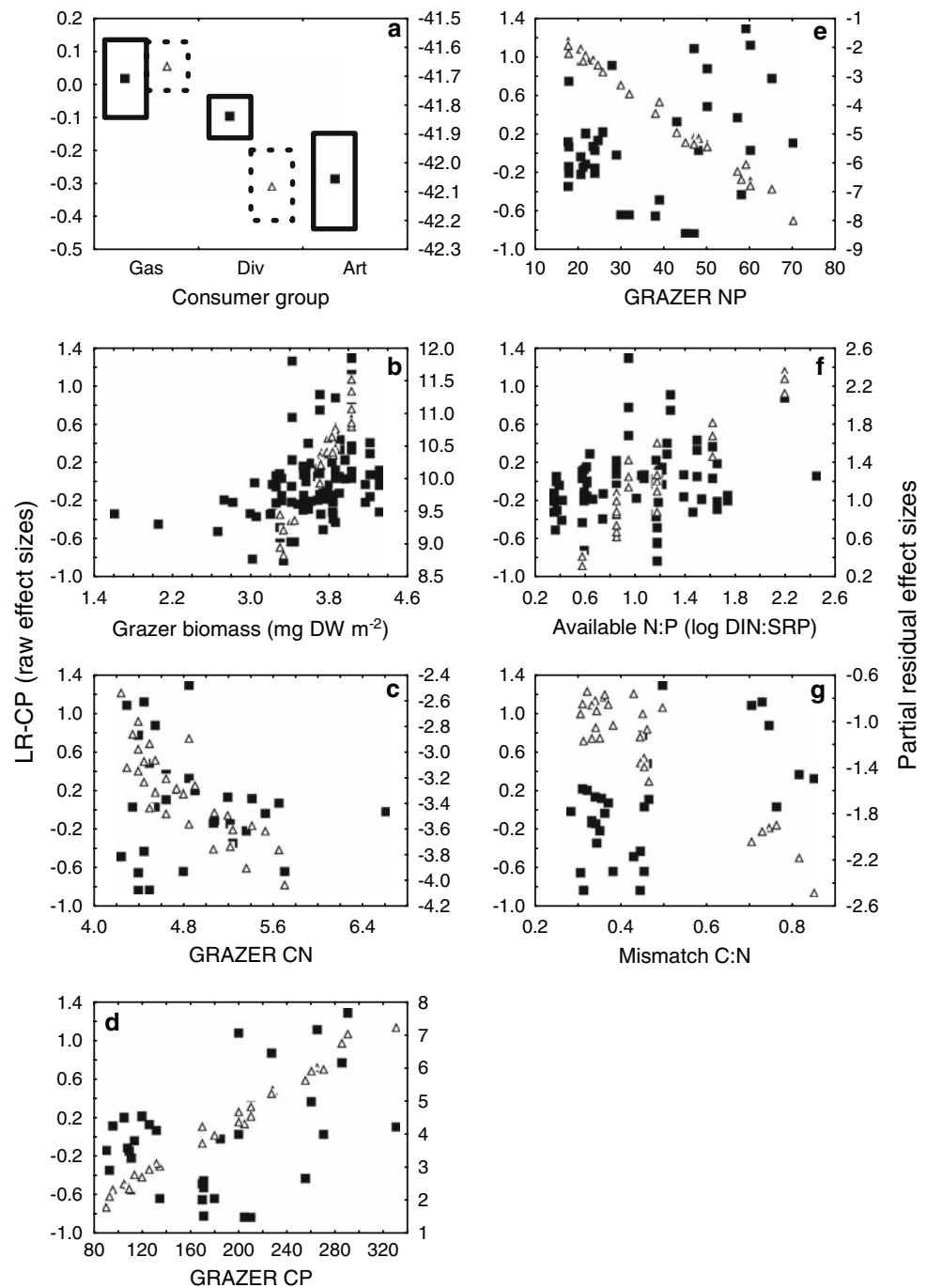

Discussion

This meta-analysis of the experimental evidence on stoichiometric constraints in grazer–periphyton interactions revealed significant effects of grazer presence on periphyton stoichiometry and identified which factors correlate to the indirect effects of grazer presence on periphyton nutrient stoichiometry. Across the different experimental setups, consumer groups and habitats, the presence of grazers increase the nutrient content of periphyton as both LR-CN and LR-CP were significantly negative. As the average effect size for C:N was larger than for C:P, N-content was enhanced more than the P-content, resulting in a positive average LR-NP, which however did not differ significantly from zero. Thus, grazer presence significantly increased the N- and P-content of the periphyton across these studies. Moreover, grazer presence also altered the ratio between N- and P-content, but not in a consistent manner across studies. Between experiments, LR-NP could vary from strongly negative to strongly positive, but the range of

Fig. 5 Bivariate plot of grazer effect sizes on periphyton N:P (LR-CP) for different consumer groups (**a**) (*Gas* gastropods, *Div* diverse, *Art* arthropods) and across gradients of **b** grazer biomass, **c** grazer C:P ratio, **d** grazer effects on periphyton biomass (LR-B), and **e** C:P mismatch between grazer and periphyton. *Symbols* as in Fig. 3
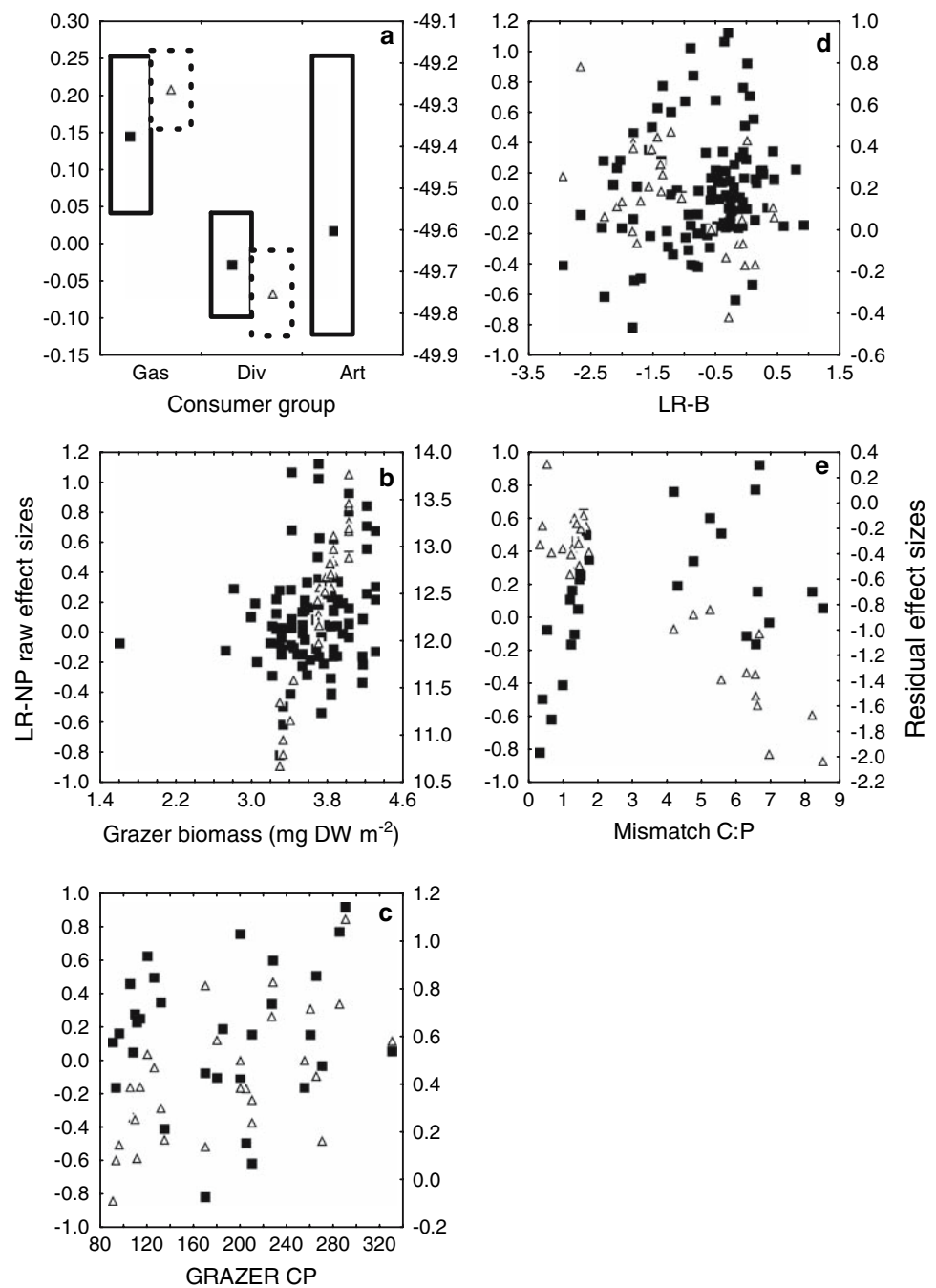
effect sizes on N:P was similar to that for LR-CN and LR-CP, respectively (cf. Fig. 2).

The analysis of 15 potential driving variables revealed some of the constraints of grazer effects on periphyton nutrient content. Univariate MA and multivariate GLM often converged upon the same driving variables, with the GLM generally reducing the number of significant predictors. The GLM analyses were able to explain more than 65% of the variance for the three effect sizes. For each of these effect sizes, both MA and GLM showed strong effects of variables related to the grazer assemblage (grazer biomass, grazer effect on peripyhton biomass, consumer group) and to the stoichiometry of the interaction (grazer nutrient content, grazer mismatch, available N:P). Other variables were significant for single analyses and single effect sizes, but the main constraints of grazer effects on nutrient content of periphyton were the structure of the grazer assemblage and grazer nutrient content (or grazer–periphyton mismatch in nutrient ratios).

The results of this analysis can shed light on the pathways of grazer effects on periphyton nutrient content (see "Pathways"). Moreover, we are able to identify the role of

grazer C:P as indicator of grazer nutrient status (see "Grazer plasticity in nutrient content"). Finally, we will discuss limitations of our approach and future research needs (see "Conclusions, caveat, and outlook").

Pathways

We outlined 3 pathways by which grazers can affect periphyton nutrient content: detritus removal, alteration of uptake rates and regeneration of nutrients by egestion and excretion (Fig. 1). The studies included in our meta-analysis did not disentangle these mechanisms. Whereas we cannot directly test validity of these different pathways, however, our analysis sheds some light on the importance of these interactions.

Detritus is often a considerable part of the diet of benthic grazers and algal biomass may only be a minor part of the biomass ingested by grazers (Hunter 1980; Lodge 1986; Cross et al. 2003). This has led to doubt about whether the stoichiometry of algae can cause changes in overall periphyton stoichiometry (Frost et al. 2002a). However, recent model calculations showed that algal stoichiometry can drive periphyton stoichiometry even if algae are a minor part of the assemblage (Frost et al. 2005b). The results of this meta-analysis indicate that it is unlikely that detritus removal by the grazers is the sole source of altered periphyton stoichiometry for two reasons. First, as detritus is mainly C-rich and poor in N and P, we would expect that periphyton C:N and C:P would be affected more strongly than periphyton N:P. This was not the case as LR-NP was similar to LR-CN and LR-CP in magnitude and range (cf. Fig. 2). Although LR-NP was not significantly different from 0 across all studies, it showed a range from a 56% reduction to a 205% increase. Second, if detritus removal would be most important, we would not expect significant effects of stoichiometric variables such as grazer C:P or grazer–periphyton mismatch on the effect sizes, which we, however, found for all three effect sizes (cf. Table 1).

Whereas we tentatively conclude that detritus removal is not the sole cause of indirect effects of grazers on periphyton stoichiometry, it remains more difficult to differentiate between the other potentially important mechanisms. The idea that grazers affect periphyton nutrient content by altering nutrient uptake has a fairly strong underlying rationale. Intact periphyton communities have low nutrient uptake rates due to boundary effects and diffusion inhibition (Riber and Wetzel 1987; Bothwell 1989; Burkholder et al. 1990). Grazer presence often reduces the proportion of erect growing, large algae (Steinman et al. 1991, 1992; Hillebrand and Kahlert 2001) and leaves a more prostrate assemblage, which eventually has higher nutrient uptake rates (Burkholder et al. 1990; McCormick and Stevenson 1991). Few of the studies in the meta-analysis compared uptake rates

between grazed and ungrazed periphyton and measured both excretion rates and ratios of the grazers at the same time to calculate the effects of nutrient regeneration (Mulholland et al. 1991). Moreover, we can not rule out the possibility that shifts from an algae-dominated to a bacteria/protist-dominated assemblage can lead to shifts in the C:N:P ratios of the entire periphyton assemblage. Grazers can have different effects on different components of the living biota within the periphyton community (Hillebrand et al. 2002) and it is sometimes assumed that bacteria have different C:N:P ratios than algae, although evidence has to date primarily originated from work in pelagic systems (Vrede et al. 2002).

The third pathway is the regeneration of nutrients by grazer excretion or egestion. Again, we lack direct information from most of the studies included in our meta-analyses (but see Evans-White and Lamberti 2006). However, we have some evidence for the importance of regeneration, as all three stoichiometric effect sizes (LR-CN, LR-CP and LR-NP) were significantly affected by grazer stoichiometry and by the mismatch between grazer and periphyton stoichiometry in the univariate analyses and the GLM. These results indicate that stoichiometric constraints influence the grazer effects on periphyton nutrient content and suggest an important role of resource regeneration by the grazers, which is the most plausible pathway how grazer stoichiometry might alter grazer effects on prey stoichiometry.

This conclusion corroborates previous results on the importance of nutrient recycling by herbivores. Consumers respond to prey food quality by altering grazing rates, nutrient assimilation and growth rate (Sterner and Hessen 1994; Frost and Elser 2002b; Urabe et al. 2002; Cross et al. 2003; Fink and Von Elert 2006). Some elements are supplied at higher relative rates than they are needed, and consumers excrete these excess nutrients or—in the case of excess carbon - increase their respiration (Darchambeau et al. 2003; Anderson et al. 2005; Frost et al. 2005a). Such consumer-driven nutrient cycling is stoichiometrically constrained (Elser and Urabe 1999) and has been shown to affect available nutrient ratios in terrestrial (Carline et al. 2005) and aquatic (Vanni et al. 2002; Frost et al. 2004; Darchambeau et al. 2005) ecosystems. Models have indicated that herbivore-driven nutrient cycling can alter plant nutrient limitation (Daufresne and Loreau 2001) and plant competition (Grover 2002).

In our meta-analysis, we defined the grazer nutrient demand from its body nutrient composition. In a meta-community study with zooplankton, Hall et al. (2004) found that zooplankton body composition did not consistently explain the distribution of different species along a P-supply gradient. They concluded that body P content was only a poor predictor of the animal's nutrient demand, as this demand is also affected by the assimilation efficiency for

the different elements and respiration rates. However, consumer nutrient content was shown to correlate strongly to threshold elemental ratios which include respiration losses and assimilation efficiencies to define the ratio of two elements where the limitation switches from one to the other (Frost et al. 2006). Thus, the use of body C:N:P ratios may be well applicable to indicate nutrient demand across species.

Grazer plasticity in nutrient content

It has been an early central tenet of ES that metazoans vary little in the elemental composition of their bodies (Sterner and Elser 2002; Villar-Argaiz et al. 2002; Darchambeau et al. 2003; Fink et al. 2006). Invertebrate consumers are not strictly homeostatic in their body nutrient composition, but show variation with age, life stage and size (Sterner and Elser 2002; Villar-Argaiz et al. 2002). Nevertheless, the animals are usually much more confined in their stoichiometry than are plants (Elser et al. 2000a; Frost et al. 2005a). In the case of relatively strict homeostasis, high grazer C:P ratios represent an inherent characteristic of the grazer, indicating a higher demand for C than for P during growth (Sterner 1990). Such a grazer will thus preferentially take up C and release P from food, which would increase the relative availability of P and reduce the cellular C:P ratio in algae. This view is difficult to align with the results of our analysis, which rather shows the opposite pattern as we found negative LR-CP and LR-NP (high P-regeneration) in experiments with grazers having low C:P ratios, i.e., high P-content. This pattern suggests that high C:P might reflect physiological constraints (P-limitation) with consecutively low P-regeneration rather than adaptive low P-demand, which suggests that it is important to consider the flexible P-content of grazer body tissues.

Two sources of variation of variable body elemental stoichiometry have to be addressed for grazing animals and are included in our analysis: phylogenetic or adaptive differences in nutrient demand across species and plasticity of nutrient content within species. The former has been shown by higher C:P threshold elemental ratios for detritivores than for grazers or predators, indicating that detritivores have adapted to their low P-food and switch to P-limitation at higher C:P than do consumers adapted to high P-food (Frost et al. 2006). The latter becomes evident by seasonal and spatial variation in P-content in a number of benthic consumers (Liess and Hillebrand 2005), where the intraspecific can be as large as the interspecific variation.

We tentatively conclude that a high grazer C:P ratio does not necessarily indicate low P-demand, but might reflect grazer P-limitation as high algal C:P would in autotrophs (Hillebrand and Sommer 1999). We are aware that the evidence for this conclusion is indirect, as we cannot estimate

the importance of grazer nutrient regeneration for the total nutrient supply to the algae from the published data. However, alternative explanations such as increased grazing pressure by high P grazers (increasing algal turnover) would require a systematic relationship of grazer P-content to both N- and P-incorporation of the algae independent of nutrient recycling. To us, it seems more straightforward that a P-limited consumer with high C:P has a relatively high P assimilation efficiency and relatively low P excretion rates. This proposition is corroborated by recent results on enrichment effects on benthic consumer nutrient content (Cross et al. 2003; Fink and Von Elert 2006; Liess and Hillebrand 2006) and own observations on within-species variability of grazer nutrient content (Liess and Hillebrand 2005).

While herbivores generally have lower variability in their nutrient content than their plant prey (Evans-White et al. 2005; Liess and Hillebrand 2005; Fink et al. 2006), our results suggest that there is the need to acknowledge their elemental flexibility in stoichiometric frameworks in order to understand indirect trophic interaction between herbivores and plants. This implies both the inclusion of flexible consumers in stoichiometric models (Grover 2003) as well as evaluating why consumers can maintain lower flexibility in nutrient content than their plant prey (Logan et al. 2004).

Conclusions, caveats and outlook

We present a general meta-analysis on the stoichiometry of grazer–periphyton interactions, including more than 100 experiments covering different experimental approaches and grazer groups. Most studies on ES so far used single consumer–prey species interactions within a very limited subset of organisms, focusing much on phytoplankton and *Daphnia*. We can conclude from our analysis that, across systems, grazed periphyton has a higher N- and P-content than ungrazed periphyton and that its N:P ratio tends to be higher. Moreover, we found that the magnitude and sign of the grazer effects on periphyton nutrient content depended mainly on the stoichiometry of the grazers, their biomass, and grazing effect size. Some of our results on N:P stoichiometry of nutrient regeneration require the assumption of flexible nutrient content of herbivorous animals. Including this flexibility in stoichiometric theory and analysing the constraints of this flexibility within species over time will be a primary research need for the future.

Whereas the advantages of our study are an increase in generality and a strong quantitative meta-analysis approach, the major disadvantage is a lack of direct control since we did not manipulate the N:P ratios in the different compartments independently. Rather we rely on statistical properties of the published experiments. Thus, we cannot

exclude the possibility that the observed changes in effect size come from another variable which we were not able to obtain from the primary publication sources. However, such a covariant factor would have to be highly influential to produce the strong patterns we observed across experiments. In addition, we lack direct observations of changing grazer nutrient content over time. It would have been preferable to have information on the within-experiment flexibility of grazer body composition, but this information was not available from the published studies. Instead we rely on between-experiment comparison to infer the flexibility of grazer nutrient content. This approach integrates over both sources of variation in body stoichiometry, the inter-specific differences and the intraspecific flexibility (see above). Again, the consistency of the findings and their corroboration of hypotheses assuming flexibility in grazer stoichiometry make us confident that these results have a strong generality across benthic aquatic systems. Conducting experimental studies on temporal changes in consumer and prey stoichiometry is another obvious future research need.

Another recurrent gap in the grazer–periphyton experiments is the lack of data about actual nutrient uptake and nutrient recycling. Information on the actual fluxes between trophic levels would allow disentangling pathways and give a more direct measure of how body nutrient content, food quality and excretion correlate. Little information is available for benthic grazers except for some exceptional studies (Mulholland et al. 1991) or special interactions such as caddis-flies and algae on their cases (Kahlert and Baunsgaard 1999). We thus propose experimental studies filling this gap by measuring the effects of grazers on periphyton and quantifying the nutrient fluxes involved.

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