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The combined effects of water level reduction and an increase in ammonia concentration on organic matter processing by key freshwater shredders in alluvial wetlands

ARNAUD DEHEDIN, CHAFIK MAAZOUZI, SARA PUIJALON, PIERRE MARMONIER and CHRISTOPHE PISCART

UMR5023 Ecologie des Hydrosystèmes Naturels et Anthropisés, Université de Lyon, Université Lyon 1, ENTPE; CNRS, 6 rue Raphaël Dubois, Villeurbanne 69622, France

Abstract

In a global change context, the intensity and the frequency of drastic low flow periods or drought events will most likely increase to a substantial extent over the coming decades, leading to a modification in the abiotic characteristics of wetlands. This change in environmental parameters may induce severe shifts in plant and animal communities and the functioning of ecosystems. In this study, we experimentally estimated the effect of drought and the accumulation of ammonia (NH_3) on the feeding activities of three generalist macroinvertebrates (i.e. *Gammarus pulex*, *Gammarus roeselii* and *Asellus aquaticus*) on three types of organic matter: leaves of *Berula erecta* growing in submerged conditions, leaves of the same species growing in emerged conditions and dead leaves of *Alnus glutinosa*. We observed a modification in the biomechanical and stoichiometric characteristics of the plants as a result of the emersion of the aquatic plants. This shift produced a substantial decrease in organic matter recycling by invertebrates and in their associated physiological ability (i.e. the energy stores of the animals) to face conditions associated with environmental change. Moreover, the accumulation of NH_3 amplified the negative effect of emersion. This snowball effect on invertebrates may profoundly modify the functioning of ecosystems, particularly in terms of organic matter production/degradation and carbon mineralization.

Keywords: *Asellus aquaticus*, feeding activity, *Gammarus pulex*, *Gammarus roeseli*, leaf litter breakdown, macrophyte emersion

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Introduction

In a context of climate change and increasing water usage (e.g. irrigation, drinking water), the intensity and the frequency of drastic low flow periods or drought events will most likely increase substantially over the coming decades (Arnell, 1999; Beniston *et al.*, 2007; IPCC, 2007). Increases in the duration of low flow periods will modify the abiotic (e.g. temperature, nutrient concentrations, eutrophication) characteristics of wetlands, inducing shifts in plant and animal communities (Scheffer *et al.*, 2001; Blenckner & Hillebrand, 2002; Greig *et al.*, 2012).

Drought first induces direct habitat changes according to the permanence of water flow and the disappearance of the water (i.e. emergence or desiccation) and induces several changes in the species composition of the plant community according to the preferences of individual species for terrestrial/aquatic conditions (Bornette & Puijalon, 2011). If exposed to emersion, certain aquatic plant species also show numerous phenotypic changes in morphological, physiological and anatomical traits (Robe & Griffiths, 1998; Li *et al.*, 2011). These phenotypic changes enable these aquatic plant species to tolerate changes in abiotic conditions induced by drought (e.g. the availability of water, light, inorganic carbon and nutrients) and also by mechanical constraints, e.g. high gravitational forces and wind-induced forces (Usherwood *et al.*, 1997; Niklas, 1998; Puijalon *et al.*, 2008). In contrast with the plant community, aquatic animals are less able to withstand emergence, and the resilience of the community is based primarily on the ability of the animals to recolonize the habitat from refuge areas (Bonada *et al.*, 2007; Dole-Olivier, 2011). Drought also increases the concentrations of dissolved substances and causes the deterioration of water quality, e.g. dysfunction in nutrient recycling and eutrophication (Delpa *et al.*, 2009). Plants and macroinvertebrates show contrasting responses to solutes (Kefford *et al.*, 2012) and to eutrophication (Greig *et al.*, 2012). Due to this diversity of response, predictions of the effects induced by global change are more complex and are dependent on local conditions (Boyero *et al.*, 2012).

Correspondence: Christophe Piscart, tel. +33 (0)4 72 44 82 69, fax +33(0)4 72 43 11 41, e-mail: christophe.piscart@univ-lyon1.fr

The consequences of these shifts may produce severe changes in the dynamics of organic matter (De Deyn *et al.*, 2008; Woodward *et al.*, 2012). Indeed, aquatic ecosystems are based on two types of primary resources: autochtonous production (e.g. algae, macrophytes) and allochtonous production by riparian vegetation, e.g. litter derived from fallen leaves (Cummins, 1974; Vannote *et al.*, 1980). The effect induced by global changes on these types of primary production may therefore impact all the dependent ecosystem functions. For example, the self-supporting morphology developed by plants in response to drought is directly related to the nature, proportion and spatial distribution of tissues in plant organs and to allometric relations between height and stem diameter (Golibert, 1989; Niklas, 1992, 1998; Speck *et al.*, 1996; Schulgasser & Witztum, 1997). Supporting tissues (e.g. sclerenchyma or collenchyma) provide structural support and mechanical resistance in plant shoots and are characterized by modified, thickened cell walls (either lignified or cellulosic), leading to an increase in the dry matter content of plant shoots (DMC; Garnier & Laurent, 1994). These changes in plant structures and mechanical properties may have significant effects on the feeding rate of invertebrates and on organic matter recycling. Several previous studies have revealed the important role of leaf mechanical properties in plant resistance to herbivory (Read & Stokes, 2006; Sanson, 2006). Mechanical resistance, provided, for instance, by high force and work-to-fracture, generally results in lower levels of herbivore damage. Structurally robust leaves are indeed consumed less by herbivores and shredders (Choong, 1996; Read & Stokes, 2006; Sanson, 2006; Ratnarajah & Barmuta, 2009). In the same way, changes in the concentrations of nutrients in water may modify the conditioning of allochtonous leaf litter by microorganisms (Suberkropp & Chauvet, 1995), which is essential for the consumption of leaf litter by shredders (Gessner & Chauvet, 1994). Increases in nutrient concentrations are also known to have an impact on the biomass of shredders and consequently on the recycling of organic matter (Piscart *et al.*, 2009). For example, Dehedin *et al.* (2013) have clearly shown the impact of ammonia on the physiology and the survival rate of crustacean shredders. Moreover, ammonia frequently increases in wetlands during flow reduction or drought (Baron *et al.*, 2009; Dai *et al.*, 2012) because of the decrease in the available water volume and dissolved oxygen concentrations (Lefebvre *et al.*, 2004) and throughout the year in response to the agricultural practices in the surrounding areas (Piscart *et al.*, 2009).

The aims of this study were to experimentally evalu-

generalist macroinvertebrates on three types of organic matter. The first two types of organic matter were obtained from the same species of macrophyte (*Berula erecta*). This plant was potentially affected by decreases in the water level and was analysed under two different growth conditions. The leaves of plants growing in submerged conditions were contrasted with the leaves of the same species growing in emerged conditions. To test the consequences of the increase in ammonia concentration throughout the year and because macrophytes are primarily available during the spring and summer, we also used alder leaves (*Alnus glutinosa*) to represent the typical food resource available to aquatic shredders during the autumn and winter. We hypothesized (H1) that the mechanical resistance of *B. erecta* leaves growing in emerged conditions would be higher than that of leaves growing in submerged conditions due to the production of strengthening tissues under terrestrial conditions to develop a self-supporting growth form (Golibert, 1989; Usherwood *et al.*, 1997). We also hypothesized (H2) that changes in the mechanical and chemical characteristics of the plants would affect the feeding rate of the three widely distributed generalist freshwater shredders examined in the study, with a decreasing degree of palatability due to differences between the properties of aquatic leaves (considered easier to consume) and terrestrial plants (considered more resistant). Moreover, to assess the effect on the organic matter resource of increased nutrient concentrations and of the interaction of these increased concentrations with a decrease in the water level, we measured the feeding rates of the three shredders on the three types of leaves along a gradient of increasing ammonia (NH_3) concentration. We thus hypothesized (H3) that a decrease in the feeding rates of the shredders would occur due to the negative effects on the physiology of the shredders of the increase in NH_3 concentration. Finally, as stress imposes energetic costs due to the need for physiological defences and thus generally induces a decrease in activity and a decrease in the resources stored in the body (Maazouzi *et al.*, 2011), we hypothesized (H4) that the combined effect of the decrease in the quantity of food ingested and the energetic costs of the physiological responses to ammonia (Maltby, 1995) would enhance the decrease in the body stores of the shredders (measured by the body content of glycogen).

Materials and methods

Selection of plant and animal species

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Materials and methods

Selection of plant and animal species

Berula erecta (Hudson) Coville is a perennial stoloniferous species. It forms a rosette of petiolated dissected leaves and measures 30–100 cm in height. The species colonizes a large

range of trophic conditions (Amoros *et al.*, 2000) and shows a high degree of variability for many morphological traits, such as size, leaflet number or leaf morphology, according to the nutritional conditions to which it is exposed (Puijalon *et al.*, 2007). *Berula erecta* is able to grow in emerged as well as in submerged conditions. *Alnus glutinosa* (L.) Gaertn is a terrestrial riparian plant found commonly in wetlands. Its leaves are consumed primarily by aquatic invertebrates after the litter falls into streams during the winter (Boyero *et al.* 2012). These two types of organic matter (i.e. macrophyte tissue and leaf litter) are not consumed simultaneously. Macrophyte is primarily consumed during the spring and summer, whereas leaf litter is primarily consumed during the autumn and winter. By studying the consumption of these forms of organic matter by shredders, we are able to estimate the potential impact of the water level reduction and the increase in ammonia concentration throughout the year.

The three freshwater shredders used in our experiments were the amphipods *Gammarus pulex* (Linnaeus 1758) and *Gammarus roeselii* (Gervais 1835) and the isopod *Asellus aquaticus* (Linnaeus 1758). These omnivorous crustacean species are widely distributed in rivers and wetlands, where they are the most important shredders (in terms of biomass) participating in the recycling of organic matter (Piscart *et al.*, 2011b).

Study site and specimen collection

All specimens (i.e. plants and animals) were sampled from a wetland adjacent to the Rhône River (45°48′N, 05°09′E). At this site, the NH_3 concentration in the water ranged seasonally between 0.002 mg L^{-1} (in the spring) and 0.043 mg L^{-1} (in the summer). Several individuals of *B. erecta* were collected and placed in 20 L tanks filled with dechlorinated tap water supplied with nutrients under different growing conditions (submerged and partially emerged) 2 months before the experiment at 18 °C and under an 18 : 6 h light : dark cycle.

Animals were sampled on different dates corresponding to the natural availability of the food resources to avoid any seasonal effect on the feeding activity of the animals (Boiché *et al.*, 2010). To study the consumption of macrophytes, animals were sampled at the end of the summer, when the plant biomass is highest. For the alder leaf litter breakdown study, animals were sampled in the winter after the abscission of the

In this study, they were used to measure the specific punch strength and the specific work to punch. The tests were performed on a universal testing machine (Instron 5942; Instron SAS, Canton, MA, USA) with a home-made device consisting of a flat-ended cylindrical steel rod (punch, ∅ 2.0 mm) mounted on the moving head of the testing machine and a stationary base with a sharp-edged hole with a clearance of 0.1 mm (following Aranwela *et al.*, 1999; Onoda *et al.*, 2008). The punch was set to pass through the hole without any friction. The punch moved downwards at a constant speed of 10 mm s^{-1}.

The tests were performed on mature leaves immediately after plant collection. For each condition, the test was performed on 20 randomly chosen leaflets. The leaflets were positioned to avoid primary and secondary veins where possible. The leaf thickness was measured with a digital thickness gauge (± 0.01 mm), avoiding major veins. The load applied to the leaflet (N) and the displacement (m) were recorded simultaneously with a frequency of 10 Hz.

The force–displacement curve was used to calculate the following quantities (Aranwela *et al.*, 1999; Read & Sanson, 2003):

The specific punch strength, F_s (N m^{-2} mm^{-1}, punch strength per unit leaf thickness), was calculated as follows:

$$F_s = (F_{max}/A)/T, \quad (1)$$

with F_{max} the maximum force (N), A the area of the punch (m^2) and T the leaf thickness (mm).

The specific work to punch, SW (J $m^{-2}m^{-1}$, work to punch the leaf per unit leaf thickness), was calculated as follows:

$$SW = (W/A)/T, \quad (2)$$

with W (J) the total work, calculated as the area under the force-displacement curve, A the area of the punch (m^2) and T the leaf thickness (mm). After the tests, the leaflets and alder leaves were dried (70 °C, 48 h), and the dry mass content (DMC) was calculated as the ratio between the dry and fresh masses.

Stoichiometric analysis of plant resources

Stoichiometric parameters are known to influence the feeding rates of animals on macrophytes (Boiché *et al.*, 2010) and on leaf litter (Webster & Benfield, 1986). To measure the elemental ratios of C, N and P, three replicates of dry material were randomly selected for each type of organic matter. Total empty all material from their guts.

Biomechanical measurements of plant resources

Punching tests ('punch and die' tests) were used to measure the force required to punch a hole through the leaf lamina.

respectively, $N = 3$). The total organic phosphorus (P) content was determined with the molybdenum blue method as described by Murphy & Riley (1958), adapted to plants. All elemental ratios were expressed as a percentage of the total dry weight, then reported as molar ratios: the C : N, C : P and N : P ratios.

Measurement of the feeding rate of shredders

The feeding rate of the three shredders was measured according to the three types of organic matter (submerged or emerged *B. erecta*, alder leaf litter) along a gradient of increasing ammonia concentrations. This factorial design allowed us to test the effects of the species, of the type of organic resource, of the ammonia concentration and of their interactions. For each condition, 20 randomly chosen individuals were placed individually in 5 cm diameter plastic cups with 30 mL of water at 18 °C under a 12 : 12 h light : dark cycle. Two leaflets of *B. erecta* or two disks (diameter 15 mm) of stream-conditioned (21 days) alder leaves were furnished to each animal. Only plant materials without any signs of grazing or necrosis were used.

The experiments were performed with sublethal concentrations of NH_3 (< 96 h LC_{50}), as previously established (Dehedin *et al.*, 2013). The nominal concentrations were prepared by dissolving the required amount of ammonium chloride (NH_4Cl) in preestablished volumes of dechlorinated tap water for a final concentration of 0.5, 1, 1.5 and 2 mg NH_4 L^{-1} for the two *Gammarus* species and 1, 5, 10 and 15 mg NH_4 L^{-1} for *A. aquaticus*. The equivalent NH_3 concentrations at 18 °C (0.09, 0.18, 0.27, 0.36 mg NH_3 L^{-1} and 0.18, 0.93, 1.82, 2.74 mg NH_3 L^{-1} respectively) were computed according to the water temperature and pH (Emerson *et al.*, 1975). The feeding rates were measured as the dry weight loss of leaf fragments after 5 days and then reported per animal body dry weight per day, i.e. mg mg^{-1} day^{-1} (Piscart *et al.*, 2011a).

Ergosterol content of alder leaves

Fungal biomass was not measured on fresh *B. erecta* leaves, where it is extremely low, but only on conditioned alder leaves, where fungal biomass plays a crucial role in leaf palatability (Gessner & Chauvet, 1994). The fungal biomass was assessed through the content of ergosterol (Gessner & Chauvet, 1993). Leaf material was freeze dried and weighed to the nearest 0.1 mg, and lipids were then extracted two times successively with methanol (25 : 1 v/w) for 15 min followed by 15 min of sonication. The extracts were concentrated in pure ethyl acetate to a final concentration of 20 mg mL^{-1} of extracts, and the ergosterol was quantified by HPLC DAD (Agilent 1200 series, Agilent Technologies Inc., Santa Clara, CA, USA). The separations were performed with a Kromasil reverse-phase

from Sigma-Aldrich (St Quentin Fallavier, France). Assays were performed on an Uvikon 940 recording spectrophotometer (Kontron, Zurich, Switzerland) at 25 °C. The glycogen content of each animal was then given as μmol of glycosyl per gram of body dry weight.

Statistical analyses

The statistical comparisons of the biomechanical and stoichiometric characteristics among the three types of organic matter were performed with a one-way ANOVA. The comparisons of both the feeding rates and the glycogen content of the animals were performed with a three-way factorial ANOVAs with the type of leaf, the shredder species and the NH_3 concentrations as fixed factors. Because the changes in glycogen body concentrations showed no statistical differences over a 5 day experiment, additional linear regressions were performed to test the correlation between the mean values of glycogen content and the NH_3 concentrations. The differences in the ergosterol content of alder leaves relative to the NH_3 concentration were tested with a one-way ANOVA. For all tests, Tukey HSD tests were used for pairwise comparisons. All statistical analyses were performed using Statistica 7 (Statsoft^{TM}, Tulsa, OK, USA) with a significance level of $P < 0.05$.

To study the correlation between the glycogen content and the feeding rate, we used the normalized mean values of the feeding rates of the animals ($n = 10$) and of their glycogen content ($n = 10$) for each ammonia concentration. These measures were normalized for each type of leaf using the following equation:

$$X_{\text{normalized}} = \frac{(X - X_{\min})}{(X_{\max} - X_{\min})} \qquad (3)$$

where $X_{\min}$ and $X_{\max}$ are the minimum and the maximum values, respectively, of the feeding rates and glycogen concentrations among all shredder species for each type of leaf. The relationship between the normalized feeding rates and glycogen contents was then tested with a linear regression to evaluate the impact of the reduction in the feeding rates on the stored glycogen.

Results

Biomechanical and stoichiometric characteristics of plant leaves

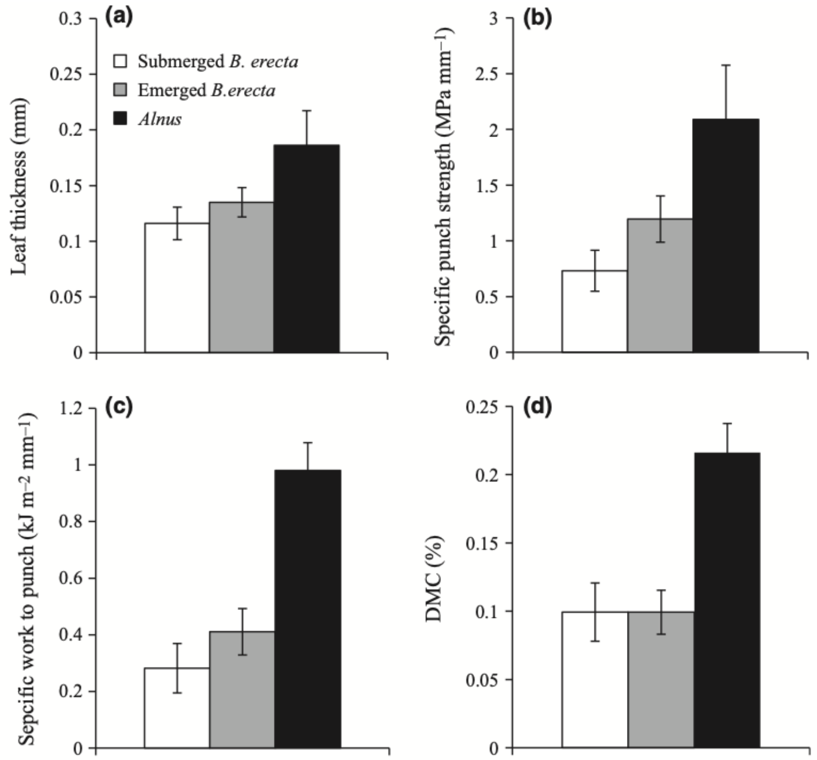

Fig. 1 Mechanical characteristics of leaves of submerged and emerged *Berula erecta* and *Alnus glutinosa* (mean ± SD, $n = 20$).

$F_{2,57} = 49.42$, $P = 0.78$), whereas the DMC of the alder leaves was significantly higher (Fig. 1d, 0.21 ± 0.02%, $F_{2,57} = 49.42$, $P < 0.001$).

We observed a similar pattern in the C : N ratios, which increased from the submerged *B. erecta* to the alder leaves (Fig. 2). The C : N ratio of the submerged *B. erecta* leaves (9.46 ± 0.03), was lower than that of the emerged leaves (14.16 ± 0.19), which was similar in value to the C : N ratio recorded for the alder leaves (Fig. 2, $P < 0.005$). These changes were explained primarily by a decrease in the N content (Table 1, ±41%), whereas the C content remained relatively stable (± 11%). However, the total phosphorus content of the submerged leaves was much lower than that of the emerged leaves ($F_{2,9} = 45.16$, $P = 0.003$), resulting in higher N : P and C : P ratios. In all cases, the leaves of *B. erecta* showed lower N : P and C : P ratios than the alder leaves, which contained a smaller amount of phosphorus ($F_{2,9} = 45.16$, $P < 0.001$).

than those of *G. roeselii* and *A. aquaticus* ($F_{4,532} = 29.71$, $P = 0.040$ and 0.034 respectively), but no difference was observed for alder leaves ($P > 0.1$). Moreover, the submerged leaves of *B. erecta* were consumed significantly more than the emerged ones ($F_{2,532} = 197.54$, $P < 0.001$) and the alder leaves were consumed less than the submerged or emerged leaves of *B. erecta* ($F_{2,532} = 197.54$, $P < 0.001$ and 0.036 respectively).

The NH_3 concentration significantly affected the feeding rate of the shredders ($F_{4,532} = 6.18$, $P < 0.001$), with a strong interaction between NH_3 and species ($F_{8,532} = 4.20$, $P < 0.001$) and type of leaf ($F_{8,532} = 2.90$, $P = 0.004$). For the submerged leaves of *B. erecta* (Fig. 3a), the feeding rates of *G. pulex* and *A. aquaticus* at intermediate NH_3 concentrations increased by 48.3% and 150.7%, respectively, and decreased substantially at higher concentrations only for *G. pulex* (by 27.7%). The feeding rate on the submerged leaves of *G. roeselii* was negatively correlated with the NH_3 concentration ($P = 0.035$). For the emerged leaves of *B. erecta* (Fig. 3b),

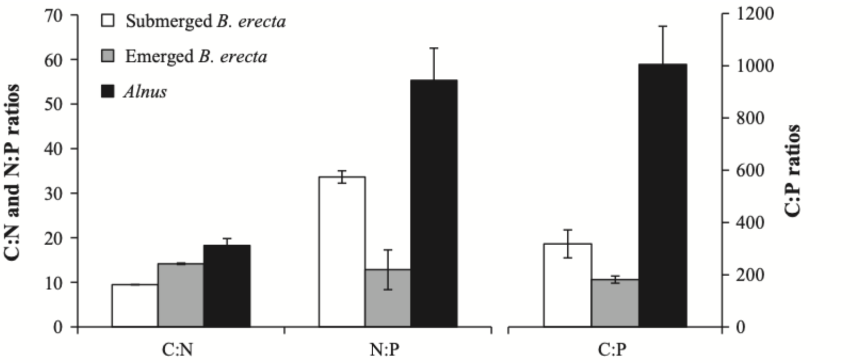

Fig. 2 Elemental composition of submerged and emerged *Berula erecta* and *Alnus glutinosa* leaves (mean ± SD, n = 3).

Table 1 Elemental composition of the tree resource types (mean ± SD, n = 3)

Resource type	%C	%N	%P
Submerged *Berula erecta*	40.69 ± 0.18	4.30 ± 0.03	0.13 ± 0.01
Emerged *Berula erecta*	36.02 ± 0.54	2.54 ± 0.01	0.20 ± 0.01
Alnus leaf litter	48.23 ± 2.13	2.65 ± 0.23	0.05 ± 0.02

($P < 0.050$). The ergosterol concentration of conditioned alder leaves decreased significantly above the threshold of 0.27 mg NH_3 L^{-1} ($F_{4,20} = 4.48$, $P = 0.010$).

Glycogen content

The glycogen content of the shredders (Fig. 4) differed significantly among species ($F_{2,532} = 89.32$, $P < 0.001$) and was significantly higher in the animals fed with the submerged leaves of *B. erecta* than in the animals fed with the emerged leaves or the alder leaves ($F_{2,532} = 29.52$, $P < 0.001$). These differences were particularly significant for *A. aquaticus*, which exhibited a glycogen content two times higher than the amphipods when fed with macrophytes.

The NH_3 concentration significantly affected the glycogen content of the shredders ($F_{4,532} = 4.59$, $P = 0.001$), with a strong interaction between NH_3 and species ($F_{8,532} = 2.30$, $P = 0.019$) but not with the type of leaf. For the submerged leaves of *B. erecta* (Fig. 4a), the glycogen content of *G. pulex* did not correlate with the NH_3 concentration ($R^2 = 0.07$, $P = 0.33$). However, the glycogen content was negatively correlated with NH_3 for *G. roeselii* ($R^2 = 0.87$, $P = 0.001$) and *A. aquaticus* species were negatively correlated with the NH_3 concentration ($R^2 > 0.67$, $P < 0.045$).

We observed positive correlations between feeding rates and the glycogen content for both amphipods, whatever the type of leaf (Fig. 5, $R^2 > 0.61$, $P < 0.059$, considered significant), whereas for *A. aquaticus*, this correlation between feeding rates and glycogen was only observed with alder leaves (Fig. 5, $R^2 = 0.66$, $P = 0.047$).

Discussion

Biomechanical and stoichiometric characteristics of plant leaves

Our results support our first hypothesis (H1) by highlighting clear differences in mechanical and chemical characteristics between emerged and submerged leaves of *B. erecta* and alder leaves. The leaflets of *B. erecta* produced under emerged conditions were thicker, consistent with previous studies performed on aquatic species producing both emerged and submerged leaves (e.g. Boeger & Poulson, 2003). The differences between both conditions in mechanical properties corrected for leaf thickness (specific punch strength and specific work to punch 65% and 45% higher than under submerged conditions respectively) also indicates changes in the material composition of the leaves. The leaves produced under terrestrial conditions were more robust (i.e. the leaves were stronger and required higher work to fracture) and presented biomechanical properties intermediate between the leaves produced under aquatic conditions and the leaves of terrestrial species (e.g. alder leaves).

These changes in mechanical characteristics are associated with changes in the stoichiometric ratios. The

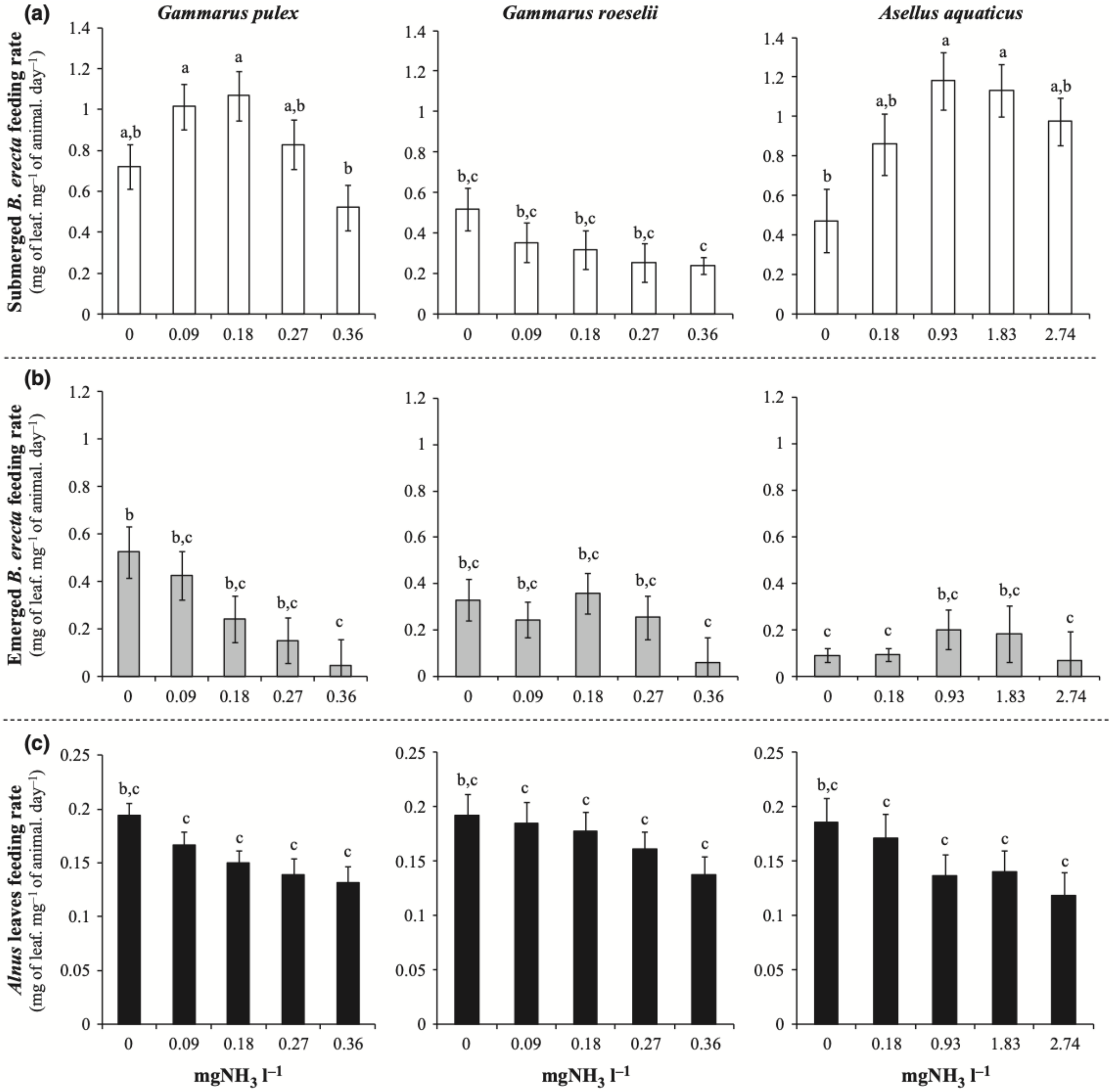

Fig. 3 Mean (± SE) feeding rate of the three shredder species (*Gammarus pulex*, *G. roeselii* and *Asellus aquaticus)* on submerged (a) and emerged (b) *Berula erecta* and *Alnus glutinosa* (c) leaves according to NH_3 concentration ($n = 20$). Note the differences in the scales of the NH_3 concentrations and in the intensity of the response for *A. aquaticus*. Significant between-condition differences (Tukey HSD test following a factorial ANOVA) in feeding rates are indicated by different letters.

i.e. supporting gravitational forces (Golibert, 1989). An increase in the phosphorus proportion in emerged tissues has also been observed in previous studies (Sytsma & Anderson, 1993) and could be a result of an increasing metabolism and photosynthesis rate in emerged leaves (Robe & Griffiths, 2000; Nekrasova *et al*., 2003) or an accumulation of organic solutes and ions for osmotic adjustment in drought-stressed plants (Pataka *et al*., 2002).

As expected, terrestrial alder leaves presented a mechanical resistance (specific punch strength and specific work to punch) higher than that of *B. erecta*, whatever the growth conditions. This result is in agreement with the characteristics of the terrestrial plant. Our results showed that the higher biomechanical parameter values for the terrestrial plants could be maintained after the abscission of the leaves. A possible explanation may be that the biomechanical

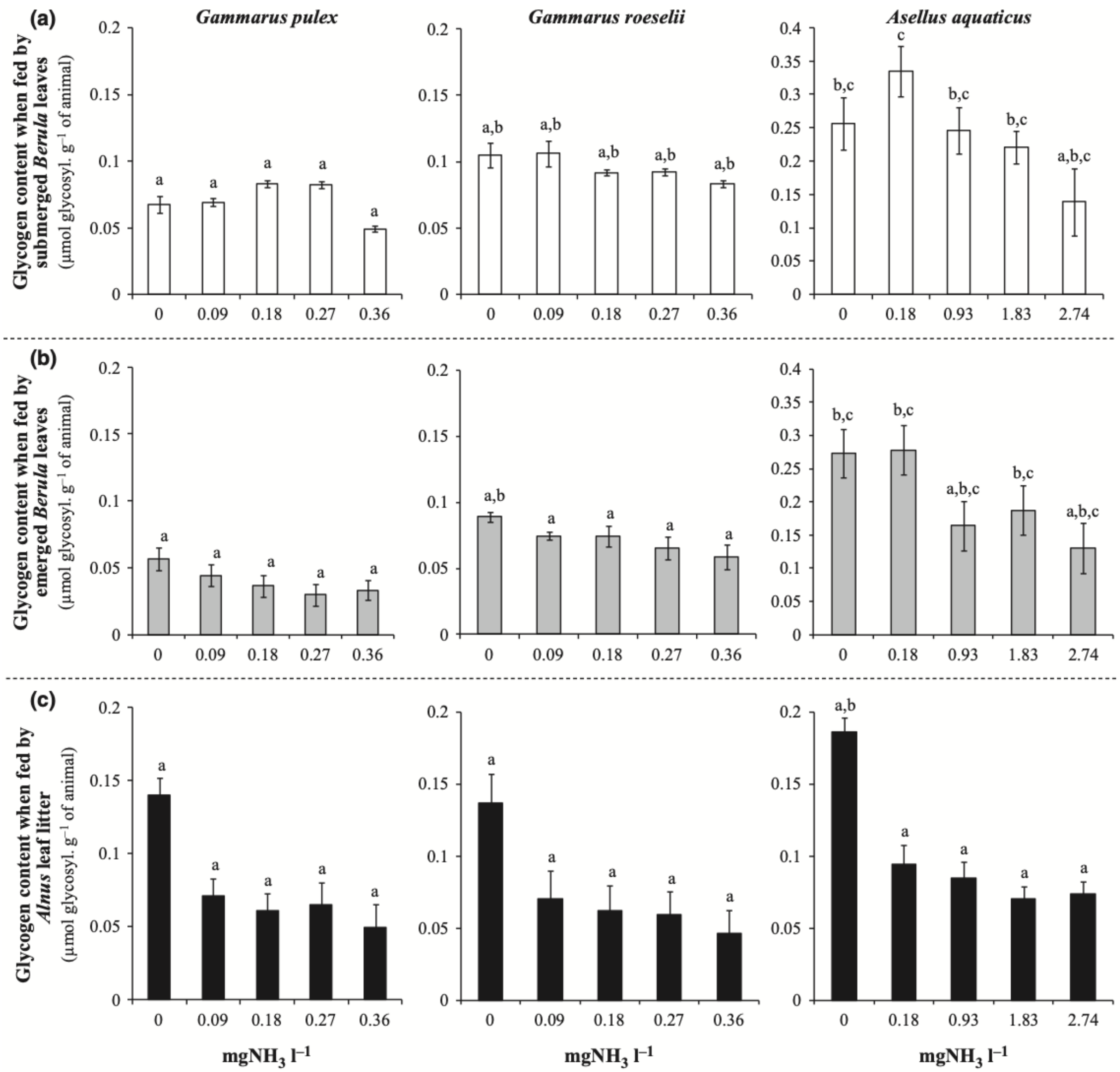

Fig. 4 Mean (± SE) glycogen content of the three shredder species (*Gammarus pulex*, *G. roeselii* and *Asellus aquaticus*) on submerged (a) and emerged (b) *Bzerula erecta* and *Alnus glutinosa* (c) leaves according to NH_3 concentration ($n = 10$). Note the differences in the scales of the NH_3 concentrations and in the body glycogen content for *A. aquaticus*. Significant between-condition differences (Tukey HSD test following a factorial ANOVA) in glycogen content are indicated by different letters.

structure of the leaves is controlled primarily by the cell wall in the sclerenchyma and the collenchyma tissues, whereas the abscission of the leaves will first affect their labile components. This hypothesis is supported by the effect of abscission on the stoichiometric ratios of leaves. Consistent with previous observations by Chapin & Kedrowski (1983), we also found low N and P contents in leaves, both indicating the predominance of carbon-rich supporting tissues.

Feeding rate of shredders

Our results showed a decreasing gradient in the feeding rate of the shredders from submerged to emerged leaflets of *B. erecta* and terrestrial alder leaves. These results support our second hypothesis (H2). In aerial conditions (i.e. emerged and terrestrial), the proportion of supporting tissues and structural elements is higher and results in a higher density of emerged organs (Golibert, 1989; Garnier & Laurent, 1994).

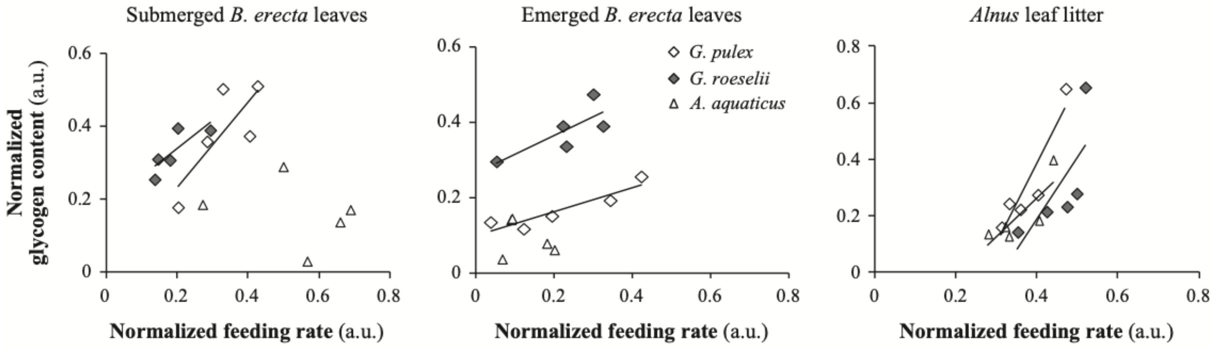

Fig. 5 Linear regression lines between the normalized mean glycogen content and the normalized mean feeding rate of the three shredder species (*Gammarus pulex*, *G. roeselii* and *Asellus aquaticus*) fed submerged and emerged *Berula erecta* leaves and *Alnus glutinosa* leaves. Only significant regression lines ($P < 0.05$) are shown.

The palatability of organic matter to shedders may be linked to two different processes. First, the mechanical characteristics of leaves modify the feeding activity of shredders. Robust leaves are generally consumed less by herbivores and shredders (Ratnarajah & Barmuta, 2009). However, we did not observe a link between the leaf DMC and the feeding rates. The DMC was previously described as an effective indicator of the relative investment in growth and defence against herbivores in macrophytes (Elger & Willby, 2003; Wong *et al.*, 2010). This anatomical trait was not relevant in our study. We did not find any intraspecific differences in DMC between the submerged and emerged leaves of *B. erecta*, whereas we observed strong variation in the feeding rate of the shredders. Moreover, changes in the feeding rate of the shredders may also be explained by changes in the C : N ratio of the organic matter. Macrophytes with a higher nitrogen content (low C : N) were generally more intensely consumed by shredders (Wong *et al.*, 2010). Our results strongly support this observation, with a higher feeding rate on the submerged leaves (with low C : N) than on the emerged leaves of *B. erecta* (with high C : N). Our study thus highlights a combined effect of the biomechanical and stoichiometric characteristics of leaves on their palatability.

In aquatic ecosystems, the palatability of macrophytes increases from the spring to the autumn according to the phenology of the plant and events associated with the growth cycle, i.e. in *Elodea* sp. (Boiché *et al.*, 2010). However, our results suggest that in ecosystems with a decrease in the water level during summer, the resulting emersion of the macrophytes might reverse this tendency by causing a decrease in plant acceptability during the low flow period. Moreover, as suggested by Boiché *et al.* (2010), plant palatability not only opportunistic consumers, such as gammarids and isopods. By reducing the development of biofilm, the emersion may therefore reduce the acceptability of the fresh leaves to the shredders.

The relationship between the biomechanical and stoichiometric characteristics of instream production (i.e. aquatic *B. erecta*) and the feeding rate of the shredders is also congruent with the biomechanical and stoichiometric characteristics of terrestrial alder leaves. Indeed, the alder leaves, which are more robust and have a higher C : N ratio, are also consumed less by shredders. Recent studies have shown that the robustness and thickness of terrestrial leaves are the primary factor explaining variation in litter decomposition and exhibit a negative effect on a global scale (Cornwell *et al.*, 2008; Onoda *et al.*, 2011). Likewise, interspecific changes in the litter consumption rate were positively correlated with the percentage of N and negatively correlated with the C : N ratio (Richardson *et al.*, 2004), which were good predictors of decomposition rates.

Effects of nutrient recycling dysfunction

Our experimental results showed that a dysfunction in nutrient recycling due to water level reduction and the resulting increase in NH_3 concentration induced an overall decrease in the feeding rate of shredders, up to 50% at the higher NH_3 concentrations. Our third hypothesis (H3) of a decrease in crustacean feeding rates with the increase in NH_3 concentration is therefore confirmed. A NH_3 concentration higher than 0.27 mg L^{-1} strongly impacts the feeding activity of shredders and may cause a modification in aquatic ecosystem functioning, e.g. in organic matter recycling (Piscart *et al.*, 2009, 2011a). These concentrations, cur-

However, in the case of the leaves that were consumed most heavily (i.e. the submerged *B. erecta* leaves), the feeding rate of *G. pulex* and *A. aquaticus* increased at low NH_3 concentrations, suggesting that these low concentrations may stimulate the feeding activity of the shredders. The stimulation of the ingestion of organic matter at a low level of disturbance may be the result of compensation, through feeding, for the physiological cost of the disturbance. Similar results have been found for the egestion rate in *Eulimnogammarus toletanus* exposed to NH_3 (Alonso & Camargo, 2004) and for the feeding rate of *G. pulex* exposed to insecticide (Crane *et al*., 1995). Another explanation of the increase in feeding rate is that the slight increase in NH_3 may stimulate bacterial and fungal growth (Suberkropp & Chauvet, 1995). This increase in the microbial biomass and activity may reduce the robustness of the leaves and increase the N and P content (Assmann *et al*., 2011) resulting in the stimulation of the feeding rate of the shredders (Gessner & Chauvet, 1994). Under these conditions, a moderate increase in nutrient concentrations with a slight accumulation of NH_3 may stimulate the decomposition of organic matter. In our short-term exposure experiment, we were not able to test this hypothesis. However, for an NH_3 concentration higher than 0.27 mg L^{-1} (i.e. the concentration at which the feeding rate of the shredders is affected), the fungal biomass, estimated by the ergosterol content in leaves, was lower than in the leaves at lower NH_3 concentrations. This decrease could partially explain the decrease in the feeding rate of the shredders for the alder leaves (Corti *et al*., 2011). Moreover, the drying regime may negatively affect the fungal community (i.e. biomass and sporulation activity; Bruder *et al*., 2011), as observed for the macrophyte, and may thus decrease the feeding rates of the shredders.

Consequences for invertebrate body stores

We observed a higher glycogen content in shredders fed with submerged *B. erecta* leaves than in shredders fed with emerged *B. erecta* leaves or alder leaves. Two processes may explain these differences in body stores. First, we found a positive relationship between the feeding rates of the two amphipod species and their glycogen content for the three types of leaves, whereas this relationship was only observed with alder leaves for *A. aquaticus*. The significant correlations between the glycogen content and the feeding rates of the shredders confirmed that the stored glycogen in these organisms may be provided by their feeding activity. In this case, the effects of NH_3 amplified the negative effect of the emergent leaves on the feeding rates of the shredders and produced a direct impact on the shredders' stored glycogen. Consequently, these effects may impact the ability of the animals to withstand environmental changes (Maazouzi *et al*., 2011). Because emergence and NH_3 interacted to affect the glycogen content of the animals, our results support our fourth hypothesis (H4). Second, crustaceans use an active NH_3 excretion mechanism to remove excessive NH_3 content from the body. This mechanism includes ATPase transporters at the gill surface (Lucu *et al*., 1989; Weihrauch *et al*., 1999). This energy-dependent mechanism might also explain the decrease in the glycogen content. The direct effect of NH_3 on the glycogen content through changes in the feeding rate is not distinguishable from its indirect effect through the excretion mechanism, but both act together to modify the ability of shredders to produce/renew their glycogen stores.

Differences were observed between the two amphipod species and *A. aquaticus*. The higher glycogen content of *A. aquaticus* in all experiments could be selectively advantageous, enabling this isopod to survive under harsh conditions. This result may explain the higher NH_3 tolerance of *A. aquaticus* relative to other crustaceans (Dehedin *et al*., 2013). In addition, the lack of a relationship between the feeding rate and glycogen body concentration observed for isopods fed with the two types of leaf of *B. erecta* also suggests strong differences between the energetic metabolism and investment strategy of *A. aquaticus* and the corresponding characteristics of the amphipod species. If the resource is of high quality (*B. erecta* leaves with low C : N), *A. aquaticus* appears to compensate for the NH_3-induced stress through an overuse of its body stores. In contrast, if the resource is of low quality (alder leaves with high C : N), feeding activity represents the major source of the energy used to compensate for the stress induced by the increase in NH_3, and a positive correlation can be observed between the feeding rate and the glycogen content of the organism.

Conclusions

Our study emphasizes that effects induced by a decrease in the water level and an increase in the NH_3 concentration may strongly impact the processing of organic matter in wetlands. This outcome may be amplified by a combined effect of climate change (i.e. the consequences of drying) and the deterioration of water quality (i.e. increases in nutrient concentrations). Modifications in the biomechanical and stoichiometric characteristics of plants induced by global change may substantially decrease the recycling of organic matter by invertebrates and the associated physiological ability of the invertebrates to withstand the effects of the environmental change. This snowball effect on the invertebrates may profoundly modify the functions of aquatic ecosystems, particularly in the case of organic matter production/degradation and carbon mineralization. Our observations and hypotheses lend further weight to the integration of the effects induced by global change on plant consumers and their contribution to the carbon cycle. Under these conditions, further field studies should quantify the contribution of each type of consumer (i.e. bacteria, fungi and invertebrates) under the pressure of global change. Moreover, our study focused on the emergence conditions experienced by the macrophyte and did not incorporate the potential interaction between the ammonia concentration and the decrease in the water level. This interaction should be studied to disentangle the consequences of global change for organic matter processes in wetlands.

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