Nutrient enrichment affects the mechanical resistance of aquatic plants

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Abstract

For many plant species, nutrient availability induces important anatomical responses, particularly the production of low-density tissues to the detriment of supporting tissues. Due to the contrasting biomechanical properties of plant tissues, these anatomical responses may induce important modifications in the biomechanical properties of plant organs. The aim of this study was to determine the effects of nutrient enrichment on the anatomical traits of two freshwater plant species and its consequences on plant biomechanical performance. Two plant species were grown under controlled conditions in low versus high nutrient levels. The anatomical and biomechanical traits of the plant stems were measured. Both species produced tissues with lower densities under nutrient-rich conditions, accompanied by modifications in the structure of the aerenchyma for one species. As expected, nutrient enrichment also led to important modifications in the biomechanical properties of the stem for both species. In particular, mechanical resistance (breaking force and strength) and stiffness of stems were significantly reduced under nutrient rich conditions. The production of weaker stem tissues as a result of nutrient enrichment may increase the risk of plants to mechanical failure, thus challenging plant maintenance in mechanically stressful or disturbed habitats.

Key words: Aerenchyma, anatomy, aquatic plant, biomechanics, mechanical stress, nutrients, phenotypic plasticity, stem density.

Introduction

Phenotypic plasticity (i.e. the capacity of a genotype to express different phenotypes in different environments, Bradshaw, 1965; Sultan, 2000) enables plants to cope with a wide variety of ecological conditions (Sultan, 2000; Santamaria, 2002). Plastic responses concern all kinds of ecologically important traits including developmental, reproductive, physiological, morphological, or anatomical ones (Sultan, 2000). Nutrient availability, particularly nitrogen, has been widely shown to affect plant growth through anatomical responses (Garnier and Laurent, 1994; Van Arendonk et al., 1997; Garnier et al., 1999; Grassein et al., 2010). Specifically, anatomical plastic responses to nitrogen enrichment are characterized by the increased production of low-density tissues (Ryser, 1996; Craine et al., 2001), rich in cellulose and proteins, such as parenchyma, both in the shoot and in the root (Poorter and De Jong, 1999). On the other hand, reduced nitrogen availability induces the production of denser tissues (Ryser, 1996; Craine et al., 2001), rich in structural carbohydrates and lignin (Garnier and Laurent, 1994; Poorter and De Jong, 1999). Moreover, silica uptake, which constitutes a structural element of certain plant species (Hodson et al., 2005), is diminished under high ammonium and nitrate concentrations (Wallace, 1989), leading to low-density tissues.

Aquatic plants present anatomical specificities, compared with terrestrial plants such as the small amount of supporting tissues, which, for many species, are concentrated in the stem in an endodermis-like structure rich in lignified cell walls and separating the
central cylinder from the cortex (Sculthorpe, 1967; Raven, 1996; Rascio, 2002). Around the central cylinder, a cortex of lacunar parenchyma (also called aerenchyma) develops and consists of a system of interconnected airspaces, allowing plant flotation and gas diffusion (Sculthorpe, 1967). This tissue facilitates gas storage and provides a lower resistance pathway for oxygen, allowing a better circulation through the plant (Colmer, 2003). Nutrient enrichment and low oxygen concentrations, often observed in nutrient-rich environments, have been shown to favour the production of low-density tissues (Puijalon et al., 2007), particularly through the development of aerenchyma in stems and roots (Hussner et al., 2009; Jampeetong and Brix, 2009).

Although many studies have investigated the morphological and anatomical plastic responses to nutrient enrichment, relatively few have measured the consequences for plant biomechanical traits (Onoda et al., 2008) and more generally mechanical resistance (i.e. the ability of an organism to endure externally applied mechanical forces). Most of these studies have focused on leaf biomechanical traits (Floater, 1997; Cornelissen and Stiling, 2006; Kerpel et al, 2006), despite the importance of the stem’s biomechanical properties which are involved in plant self-support and also in resistance to mechanical forces (Niklas, 1993, 1995; Schugasser and Witzum, 1997).

In ecosytems periodically disturbed by floods, aquatic plants may be exposed to external mechanical forces induced by floods that may cause shoot breakage and hence a loss of biomass and meristems and, therefore, a reduced fitness (Mony et al., 2011). Plant resistance to hydrodynamic forces relies on a minimization of the forces encountered and on a maximization of the resistance to breakage of plant organs (Puijalon et al., 2007). Resistance to breakage is measured by the strength, corresponding to the force that a plant fragment can withstand without suffering mechanical failure, corrected by the cross-sectional area of the fragment (Denny, 1988; Niklas, 1992). Stiffness, which is the ability to resist deformation in response to an applied force, is negatively linked with the reconfiguration ability of the plant, (i.e. changes in plant shape with increasing velocity, Vogel, 1984; Sand-Jensen, 2003) and thus to the hydrodynamic forces encountered by plants (Vogel, 1994; Sand-Jensen, 2003). Reconfiguration enables plants to alternate a configuration maximizing photosynthesis by maximizing leaf exposure to light in standing conditions, and a streamlined configuration in flowing water (Vogel, 2003). Strength and stiffness are thus key biomechanical traits involved in aquatic plant resistance to hydrodynamic forces, either through the maximization of tissue resistance or through the minimization of hydrodynamic forces.

The determination of stem strength and stiffness is complex since it depends on organ size, tissue position around the axis and tissue properties (Niklas, 1992), all these traits being closely related to stem anatomy (Onoda et al., 2008). Aerenchyma is a mechanically weak tissue providing little stiffness to plants (Niklas, 1992; Striker et al., 2007), despite reinforcement provided by sclerenchymatous diaphragms localized at the nodes of submerged stems and petioles (Sculthorpe, 1967; Sorrell and Dromgoole, 1988). Supporting tissues, sclerenchyma and collenchyma, composed of dead and living cells, respectively, are characterized by thickened cell walls. Thick cellulose cell walls present in collenchyma lead to greater strength (Kokubo et al., 1989) and lignin, present in sclerenchyma tissue, provides good stiffness and high compression strength (Evert, 2006). Silica, a hard material, may also be involved in organ stiffness (Sanson et al., 2007) by reinforcing cell walls (Scholz et al., 2010). Variations in the allocation to tissues or structural elements caused by nutrient enrichment might consequently modify the biomechanical properties of aquatic plants, particularly in the sense of a reduced mechanical resistance due to the decreased proportion of supporting tissues and an increased proportion of low-density tissues.

The aim of the present study was to determine the effects of nutrient enrichment on the anatomical traits of freshwater plants, particularly aerenchyma, and its consequences for their biomechanical properties. The hypotheses that a high nutrient supply leads to (i) decreased stem density induced by anatomical modifications and (ii) reduced mechanical resistance of the stem associated with these anatomical modifications, were specifically tested. Two freshwater plant species, Myosotis scorpioides L. and Mentha aquatica L., were cultivated under controlled conditions for both high and low nutrient concentrations. The plant response to nutrient enrichment was characterized by measuring three sets of traits. The first, ‘macroscopic traits’, that measures stem density, consisted of dry matter content (DMC=dry mass/fresh mass) and dry matter concentration (D=dry mass/organ volume). These traits were used as indirect indicators of anatomical structure. DMC described the amount of dry mass relative to fresh mass, which increased in proportion to tissues with thickened cell walls (sclerenchyma and collenchyma) and/or with the presence of heavy elements such as silica (Garnier and Laurent, 1994). D took into account volume (Shipley and Vu, 2002), which is relevant to aquatic plants due to the high proportion of air spaces in aerenchyma. The second set of traits, ‘microscopic traits’, consisted of the proportion taken up by the central cylinder area and two traits describing aerenchyma structure (average individual lacuna area and total lacuna area relative to aerenchyma area). The third set consisted of ‘biomechanical traits’ characterizing resistance to breakage (breaking force and strength) and flexibility (flexural stiffness).

Materials and methods

Plant material and sampling sites

The study was conducted on two aquatic plant species: Myosotis scorpioides L. (Boraginaceae) and Mentha aquatica L. (Lamiaceae) (Fig. 1A). They are both found under a wide range of nutrient conditions and can colonize habitats with a relatively high level of scouring due to flood disturbance (Amoros et al., 2000). One hundred individuals of each species were collected in February 2010, under totally submerged conditions, in two oligo-mesotrophic channels of the Ain River (France): Méant (05°08’04'' E, 45°48’30’’ N) and Vilette (05°16’57’’ E, 45°59’08’’ N), respectively. These channels are periodically disturbed by floods, which can generate high water velocities leading to plant breakage and uprooting (Bornette et al., 1994). Except for flooding periods, water flow in these channels is extremely low and plants were sampled under standing conditions (velocity <0.05 m s⁻¹, Puijalon et al., 2007).

Culture conditions and treatment

For each species, individuals were divided into two sets of 50 individuals. Each set was cultivated separately in one aquarium (100 × 45 × 40 cm)
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All aquaria were filled with 7 cm of sand (0–4 mm) and tap water. Temperature was controlled during the experiment (17.8 ± 0.6 °C) with a refrigeration unit and the water was aerated using air pumps. The aquaria were illuminated by neon light (5.07 ± 2.79 klx surface light with a 12 h photoperiod).

The two nutrient levels applied (low versus high) were chosen to reflect values measured in mesotrophic natural habitats (Puigalon et al., 2007). The nutrient conditions were applied to both species following plant transplantation by adding a liquid fertilizer (Miracle-Gro Liquafeed, NPK 12:4:8, Scotts Company). The quantity of fertilizer added to the aquaria at the beginning of the experiment corresponds to an increment of 1.2 mg l⁻¹ of ureic nitrogen and 0.40 mg l⁻¹ of P-PO₄³⁻ at high nutrient conditions compared with low nutrient ones. Four days after application of the treatment, concentrations of P-PO₄³⁻, N-NO₃⁻, and N-NH₄⁺ were measured by the colorimetric method using a spectrometer (Easychem Plus SystemTM). The concentrations were 24.35 versus 128.47 µg l⁻¹ for [P-PO₄³⁻], 1.94 versus 1.94 mg l⁻¹ for [N-NO₃⁻], and 4.09 versus 21.84 µg l⁻¹ for [N-NH₄⁺], for low versus high nutrient levels, respectively. To ensure that conditions other than nutrient contents were similar between the two aquaria of the same species, homogeneity was checked for temperature (17.5 ± 0.6 versus 17.7 ± 0.5 °C for M. scorpioides and 18.3 ± 0.5 versus 18.0 ± 0.7 °C for M. aquatica, for low versus high nutrient levels, respectively), light (4.9 versus 4.8 klx and 5.3 versus 5.3 klx), oxygen (6.8 versus 7.2 mg l⁻¹ and 8.2 versus 8.4 mg l⁻¹), and pH (8.2 versus 8.3 and 8.6 versus 8.4) between aquaria during the experiment.

Harvest

All individuals were harvested 10 weeks after the start of treatment. For each individual, a fragment of approximately 7 cm was collected in the basal portion of the stem to measure its biomechanical and anatomical macroscopic traits (DMC, D). As it was not possible to measure the microscopic traits on the same fragment, due to the damage caused when measuring biomechanical traits, these traits were measured on a 2 cm fragment collected next to the first one.

Measurement of anatomical traits

Macroscopic traits

Fresh mass and dry mass (obtained after drying for 24 h at 70 °C, ±0.0001 g) and fragment length (±1 mm) were measured for each fragment. These measurements were used to calculate dry matter content (DMC=dry mass/fresh mass) and dry matter concentration (D=dry mass/organ volume). Fragment volume was obtained by multiplying its length by its cross-sectional area (see microscopic traits).

Microscopic traits

Fragments were immersed for 12 h in 60% ethanol before measuring their microscopic traits. Stem cross-sections were cut by hand with a razor blade and stained with Mirande’s reagent (Deysson, 1954) which stains pecto-cellulosic walls pink and lignified elements green. Stem cross-section images were taken using an optical microscope connected to a digital camera (Fig. 1A). Two reference distances were measured for each image with an ocular micrometer in order to calibrate the images. Areas of the different main stem structures and tissues were delimited using Adobe Photoshop CS3 10.0: cross-section, central cylinder, aerenchyma, and lacunae (Fig. 1B). The surface areas of these structures were then measured using ImageJ 10.2 software after calibrating the images with the reference distance. These measurements were used to calculate the following traits (Fig. 1B):

(i) stem cross-sectional area (mm²)
(ii) central cylinder area relative to cross-sectional area, measured on a quarter of the cross-section
(iii) total lacuna area relative to aerenchyma area, measured on a quarter of the cross-section
(iv) average individual lacuna area, measured on a quarter of the cross-section (mm²)

Fig. 1. (A) Entire plant and stem cross-section, showing aerenchyma (ae) and central cylinder (cc) position for the two species and (B) anatomical traits measured; (1) cross-sectional area, (2) aerenchyma area, (3) central cylinder area, and (4) lacunar area. (This figure is available in colour at JXB online).
Measurement of biomechanical traits

The three-point bending test was used to measure the biomechanical traits of the stem (Usherwood et al., 1997; Vogel, 2005). A stem fragment was clamped over two supports 4 cm apart. The clamped zone was protected with plastic paraffin film. An increasing load was applied to the middle of the fragment until it broke and the total mass necessary to break the fragment was measured. The tests were carried out in front of a background sheet of graph paper, and filmed with a digital camera to measure stem deflection. Deflection represents the degree to which an element is displaced under a load (Niklas, 1992). The maximal distance of stem deflection was measured here. The following traits were calculated.

(i) Breaking force (N) was the maximum force that the plant stem could withstand before breaking.

(ii) Second moment of area, I (m4), quantified the amount of matter of the stem around a reference axis and depended on the cross-section geometry of the stem (Niklas, 1992). A circular cross-section was considered for M. scorpioides (I = πR4/4; R: radius) and a square cross-section for M. aquatica (I = b4/12; b: side) (Niklas, 1992). To take into account the high proportion of lacunae in stems of aquatic plants, the second moment of area was also corrected by porosity (Icor = I (1−P)). This correction made it possible to quantify the effective cross-sectional area supporting forces in bending tests (Choi et al., 2007).

(iii) Stress, σ (N m−2) measured internal forces acting within a deformable body. The stress in bending, at breaking point, called flexural strength, was obtained by dividing the breaking force per second moment of area of the body, where the forces were applied (Niklas, 1992).

\[
\sigma = \frac{My}{I}
\]  

where M was the flexural moment corresponding to the breaking force increased by the distance between fixed ends, and by a 1/8 factor; y the vertical distance between the point where force was applied to the cross-section centre (neutral axis), and I the second moment of area.

(iv) Elastic modulus, E (N m−2) measured stem stiffness per area and it was calculated by using the stress-deflection ratio

\[
E = \frac{FL}{192dl}; d: \text{deflection}
\]  

(v) Flexural stiffness, EI (N m2) measured the aptitude of a structure to resist bending. This parameter integrated elastic modulus (material property) and the second moment of area (shape property).

Statistical analyses

Analyses of covariance (ANCOVA) were used to analyse the effect of nutrient level on all anatomical traits (both macroscopic and microscopic), except average individual lacunar area. Data were log transformed to improve the normality of residuals and homogeneity of variance. For each trait, nutrient level, covariate, and interaction were added to the model. For DMC, the ANCOVA was carried out with dry mass as the dependent variable and fresh mass as the covariate. For D, dry mass was used for the ANCOVA as the dependent variable and volume as the covariate. The dependent variables tested by ANCOVA corresponding to the macroscopic traits were central cylinder area and total lacunar area, where the cross-sectional and aerenchyma areas were used as covariates. Non-significant interactions were removed to obtain the final model. When treatment effects were not significant, a simple linear regression was made. Student or Welch tests, assuming unequal variances, were made to compare average individual lacunar areas and biomechanical parameters (second moment of area, breaking force, deflection, flexural strength, elastic modulus, and flexural stiffness). All statistical analyses were performed with R 2.9.2 software (R-Development-Core-Team, 2009).

Results

All individuals survived until the harvest date, except five individuals of M. scorpioides at high nutrient levels.

Macroscopic traits

For M. aquatica, D was significantly lower at a high nutrient level, but not DMC (Table 1; Fig. 2A, 2B). For M. scorpioides, DMC and D were significantly lower at a high nutrient level (Table 1, Fig. 2A, 2B).

Microscopic traits

The M. aquatica stem is characterized by a large central cylinder, an aerenchyma cortex and collenchyma tissue at each corner of the stem. No differences were found in the proportion of collenchyma area relative to the stem cross-section between treatments (data not shown). The proportion of the central cylinder area relative to the stem cross-section was significantly lower under high nutrient conditions (Table 1; Fig. 2C). The stem of M. scorpioides is characterized by an area of aerenchyma around a central cylinder. The relative proportions of both structures (aerenchyma and central cylinder) were not significantly different between nutrient conditions (Table 1; Fig. 2C).

For M. aquatica, the total lacunar area relative to the aerenchyma area did not differ significantly between nutrient conditions.

Table 1. Effects of nutrient level on DMC, D, proportion of central cylinder and proportion of total lacunar area tested with ANCOVA. F, df and significance of selected model are presented (*P < 0.05; **P < 0.01; ***P < 0.001)

| Effect                  | DMC          | D     | Proportion of central cylinder area | Proportion of total lacunar area |
|-------------------------|--------------|-------|-------------------------------------|---------------------------------|
| Covariate               | Log dry mass | Log dry mass | Log central cylinder area | Log total lacunar area |
| Covariate (C)           | F1,16=642.7 *** | F1,16=100.4 *** | F1,16=604.8 *** | F1,16=42.66 *** |
| Nutrient level (T)      | F1,16=3.77 ns  | F1,16=13.3 *** | F1,16=5.79 *  | F1,16=0.19 ns  |
| CxT                     | F1,16=0.38 ns  | F1,16=2.69 ns  | F1,16=1.56 ns  | F1,16=2.97 ns  |
| M. scorpioides          | Covariate (C) | F1,16=252.6 *** | F1,16=50.55 *** | F1,16=68.1 *** | F1,16=281.9 *** |
| Nutrient level (T)      | F1,16=7.32 **  | F1,16=4.24 *   | F1,16=0.34 ns  | F1,16=0.1 ns   |
| CxT                     | F1,16=2.17 ns  | F1,16=2.54 ns  | F1,16=0.32 ns  | F1,16=2.02 ns  |
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**Fig. 2.** Effects of nutrient level on (A) DMC, (B) D, (C) central cylinder area relative to cross-sectional area, and (D) total lacunar area relative to aerenchyma area for *M. aquatica* and *M. scorpioides*. Points represent values and line regressions of ANCOVA; (open circles) low nutrient level; (filled circles) high nutrient level.
Biomechanical traits

The second moment of area, deflection, and modulus of elasticity of *M. aquatica* did not differ significantly between treatments (Table 2). However, the breaking force and flexural stiffness were significantly lower under high nutrient conditions, (24% and 21% lower, respectively; Table 2). Strength was also significantly lower under high nutrient conditions when it was corrected to porosity (34% lower under high nutrient conditions; Table 2). The second moment of area and deflection of *M. scorpioides* were not significantly different between treatments (Table 2). However, the breaking force, strength, elastic modulus, and flexural stiffness were significantly lower under high nutrient conditions (63, 59, 54, and 60% lower, respectively; Table 2).

Discussion

Anatomical and biomechanical responses to nutrient enrichment

In accordance with our hypotheses, the anatomical traits of *M. aquatica* and *M. scorpioides* were significantly affected by nutrient level, leading to a lower flexural strength and stiffness of plant stems under high nutrient conditions. These anatomical variations are consistent with previous studies that showed, both for terrestrial and aquatic species, a low density of plant tissues under high nutrient levels (Ryser, 1996; Craine *et al.*, 2001; Puijalon *et al.*, 2007) related to a lower concentration of structural components (Garnier and Laurent, 1994; Van Arendonk *et al.*, 1997). Low concentrations of structural components such as cellulose, leads to shoots with thinner cell walls, as well as an increasing proportion of air spaces in roots, reducing plant organ strength (Kokubo *et al.*, 1989; Striker *et al.*, 2007). Only a few studies examined both the anatomical response and its consequences on biomechanical traits and these focused mostly on leaf traits (Onoda *et al.*, 2008). To our knowledge, the present study is the first one to demonstrate the mechanical consequences of stem anatomical variations induced by nutrient enrichment.

For *M. aquatica*, nutrient enrichment led to modifications in anatomical structures (reduced central cylinder area relative to cross-sectional area and larger individual lacunae) and changes in strength (reduced flexural strength after correction to stem porosity). The DMC, which describes the proportion of structural elements by means of the cell wall to cytoplasm ratio, did not differ between treatments, suggesting that the structural elements (e.g. proportion of supporting tissue) did not differ between treatments. It can be hypothesized that the observed differences in flexural strength are due to differences in organization of the aerenchyma. Particularly, larger individual lacunar areas (but identical porosity) under high nutrient condition results in a lower perimeter to area ratio of lacunae. Lacunar strength depends on air resistance and on contouring cell wall resistance (Sculthorpe, 1967; Sorrell and Dromgoole, 1988), which is lower under high nutrient conditions, resulting in the lower mechanical resistance observed under high nutrient conditions.

The lower rigidity (EI) observed under high nutrient conditions for *M. aquatica* results from a combination of tissue rigidity (E) and distribution through the stem (I), neither parameter being significant on its own, and might be explained by differences in the central cylinder area relative to the cross-sectional area. At low nutrient levels, the central cylinder area relative to the cross-sectional area is higher, which results in a more peripheral positioning of the sustaining tissue, particularly the endodermis rich in lignified cell walls. Having supporting tissues nearer the periphery has been shown to increase stem stiffness (Sculthorpe and Witztum, 1997; Usherwood *et al.*, 1997; Ettnier and Villani, 2007): when stems are exposed to bending, the outer fibres endure maximal forces (Niklas, 1992; Vogel, 2003) thus, having more rigid tissues in a peripheral position maximizes stem rigidity.

For *M. scorpioides*, under high nutrient conditions, both DMC and D were significantly lower, suggesting a cell wall

| Table 2. Mean (±SD) and significance (*P < 0.05; **P < 0.01; ***P < 0.001) of Student’s t test (t) or Welch’s test (w) on biomechanical traits under high and low nutrient levels (l), second moment area (lcor), second moment area corrected by porosity, deflection, breaking force; α, bending stress; αcor: breaking stress corrected for porosity, E, elastic modulus; Ecor, elastic modulus corrected for porosity; EI, flexural stiffness). |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| **M. scorpioides**              | **M. aquatica** | **M. scorpioides** | **M. aquatica** |
| Low nutrient level (n=19)       | High nutrient level (n=17) | Student’s t test or Welch test | Low nutrient level (n=36) | High nutrient level (n=33) | Student’s t test or Welch test |
| I (mm³)                         | 0.57±0.27       | 0.47±0.18       | ns (t)          | 2.68±1.95       | 3.40±2.30       | ns (t)          |
| Icor (mm³)                      | 0.47±0.18       | 0.39±0.15       | ns (t)          | 2.39±1.83       | 2.96±2.11       | ns (t)          |
| Deflexion (cm)                  | 2.68±0.83       | 2.56±0.83       | ns (t)          | 2.36±0.43       | 2.22±0.57       | ns (t)          |
| Breaking force (N)              | 2.09±0.62       | 0.77±0.83       | *** (w)         | 5.35±2.03       | 5.98±2.17       | * (t)           |
| α (MPa)                         | 19.39±9.91      | 8.09±10.97      | ** (w)         | 16.96±7.55      | 12.06±12.48     | ns (t)          |
| αcor (MPa)                      | 22.88±13.92     | 9.48±5.91       | *** (w)         | 20.54±12.57     | 13.65±13.41     | * (t)           |
| E (MPa)                         | 55.66±20.85     | 25.08±28.79     | ** (w)         | 46.18±27.18     | 36.72±52.34     | ns (w)          |
| Ecor (MPa)                      | 65.43±39.36     | 29.49±15.74     | ** (w)         | 58.14±49.99     | 41.28±60.87     | ns (t)          |
| B (10⁹N m²)                     | 2.58±1.01       | 1.02±0.93       | *** (w)         | 8.27±2.88       | 6.56±3.0        | * (t)           |
modification and stems with lower mechanical resistance (lower breaking force and flexural strength) and lower stiffness. It can be hypothesized that, for *M. scorpioides*, these changes in tissue density and biomechanical traits are due to a decrease in the proportion of strengthening elements (Garnier and Laurent, 1994). These strengthening elements could be thickened cell walls (either cellulosic or lignified), but also silica. Silicone, which is used by the *M. scorpioides* family, the Boraginaceae (Hodson et al., 2005), has a high molecular mass and is absorbed by roots in its soluble form (H₂SiO₄) and polymerized into a crystallized form (SiO₂.nH₂O) in the cell walls or cytoplasm (phytolithe) (Raven, 2003). Nitrate and ammonium ions have been shown to restrain silica uptake in rice (*Oryza sativa*) by ionic competition (Wallace, 1989): a high ratio of cation (e.g. NH₄⁺) to anion uptake may induce a proton excretion, acidifying the environment, which makes silica less soluble (Wallace, 1989). As silicone provides resistance to lodging in crops (Hasan et al., 1993), a reduced silica uptake under high nutrient conditions could thus explain both the low DMC and D and the lower flexural rigidity observed in *M. scorpioides*.

Adaptive value of plant responses when exposed to multiple stresses

The responses of anatomical traits to resource availability (e.g. nutrients, oxygen, and light) enable plants to adapt to these conditions (Ryser, 1996; Van Arendonk et al., 1997; Garnier et al., 1999). Under nutrient-poor conditions, the production of dense tissues (e.g. thicker, lignin-rich cell walls,) enhances organ lifespan and nutrient conservation, whereas under nutrient-rich conditions, low-density tissues, rich in proteins and cellulose are linked to high growth rates (Ryser, 1996; Poorter and De Jong, 1999). The present results are consistent with these previous studies: the allocation to structural components is lower under high nutrient conditions.

In aquatic habitats, high nutrient levels are also frequently accompanied by low water oxygenation (Camargo and Alonso, 2006). The greater development of aerenchyma in fully submerged plants may represent an adaptation to the hypoxic conditions induced by nutrient enrichment (Hussner et al., 2009; Jampeetong and Brix, 2009; Ryser et al., 2011) as it enhances gas diffusion through the plant, thus improving the respiration rate (Rascio, 2002; Sorrell et al., 2002; Colmer, 2003). Larger lacunae offer little resistance to oxygen diffusion, facilitating its circulation under hypoxic conditions (Sorrell et al., 2002; Colmer, 2003). The responses of anatomical traits observed in the present study could therefore represent adaptations to both nutrient conditions (nutrient conservation, organ lifespan) and the indirect effects of nutrient conditions, particularly oxygen levels (gas circulation).

Under natural conditions, plants are frequently subjected to multiple environmental factors (Chapin et al., 1987; Valladares et al., 2007). In particular, in all ecosystems, plants can be exposed to external mechanical factors, for instance, induced by waves, flow or wind. In the present case, aquatic plants can be exposed to high hydrodynamic forces during floods that periodically disturb habitats (Bornette and Puijalon, 2011). Such factors may lead to plant breakage when the forces encountered by the plants exceed tissue resistance to breaking (Koehl, 1982; Schutten et al., 2005; Puijalon et al., 2011). Even if, for some species, plant fragments are able to regenerate, favouring dispersal (Barrat-Segretain et al., 1998; Barrat-Segretain and Bornette, 2000), shoot breakage induced by mechanical factors may also reduce plant fitness, due to loss of biomass and meristems (Mony et al., 2011). In addition, for *Berula erecta*, the survival and regeneration of fragments have been demonstrated to be lower for plants growing in nutrient-rich habitats, probably due to the quantity or nature of carbohydrates stored (Puijalon et al., 2008). In the present study, it has been shown that the anatomical responses of aquatic plants to nutrient enrichment lead to the production of weaker stems, which may result in a higher risk of breakage for the plants exposed to mechanical forces. Adaptive plastic response to nutrient enrichment may, therefore, incur a cost, through the production of phenotypes more vulnerable to mechanical factors, presenting a higher breaking risk and thus potentially lower survival rates. Due to its negative effect on the mechanical resistance of plant stems, nutrient enrichment could represent an indirect factor in reducing plant resistance to mechanical factors, thus challenging plant maintenance in mechanically disturbed habitats.

The responses to nutrient enrichment demonstrated in the present study under standing conditions may differ for plants growing in running habitats and encountering permanent mechanical stress induced by flow. Individuals growing in running habitats may present anatomical adaptations (e.g. increased allocations to strengthening tissues) resulting in enhanced resistance to breakage (Biehle et al., 1998; Bociag et al., 2009). Due to their partly antagonistic effects, interactive effects of mechanical stress and nutrient level lead to complex responses for morphological and anatomical traits (including stem density), which may result in reduced plant capacity to adapt to running conditions (Puijalon et al., 2007).

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References

Amoros C, Bornette G, Henry CP. 2000. A vegetation-based method for ecological diagnosis of riverine wetlands. *Environmental Management* 25, 211–227.

Barrat-Segretain M–H, Bornette G. 2000. Regeneration and colonization abilities of aquatic plant fragments: effect of disturbance seasonality. *Hydrobiologia* 421, 31–39.

Barrat-Segretain M–H, Bornette G, Hering–Vilas–Boas A. 1998. Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquatic Botany* 60, 201–211.

Biehle G, Speck T, Spatz HC. 1998. Hydrodynamics and biomechanics of the submerged water moss *Fontinalis antipyretica*: a
comparison of specimens from habitats with different flow velocities. *Botanica Acta* **111**, 42–50.

**Bociag K, Galka A, Lazarewicz T, Szmeja J.** 2009. Mechanical strength of stems in aquatic macrophytes. *Acta Societatis Botanicorum Poloniae* **78**, 181–187.

**Bornette G, Amoros C, Chessel D.** 1994. Effect of allophenic processes on successional rates in former river channels. *Journal of Vegetation Science* **5**, 237–246.

**Bornette G, Pujalson S.** 2011. Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences* **73**, 1–14.

**Bradshaw AD.** 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* **13**, 115–155.

**Camargo JA, Alonso I.** 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment International* **32**, 831–849.

**Chapin III FS, Bloom AJ, Field CB, Waring RH.** 1987. Plant responses to multiple environmental factors. *BioScience* **37**, 49–57.

** Choi D, Jeon J, Lee P, Hwang W, Lee K, Park H.** 2007. Young's modulus measurements of nanohoneycomb structures by flexural testing in atomic force microscopy. *Composite Structures* **79**, 548–553.

**Colmer TD.** 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell and Environment* **26**, 181–194.

**Cornelissen T, Stiling P.** 2006. Does low nutritional quality act as a plant defence? An experimental test of the slow-growth, high-mortality hypothesis. *Ecological Entomology* **31**, 32–40.

**Craine JM, Froehle J, Tilman DG, Wedin DA, Chapin IFS.** 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos* **93**, 274–285.

**Denny M.** 1988. *Biology and the mechanics of the wave-swept environment*. Princeton, New Jersey, USA: Princeton University Press.

**Deysson G.** 1954. *Eléments d’anatomie des plantes vasculaires*. Paris: Sedes.

**Etnier SA, Villani PJ.** 2007. Differences in mechanical and structural properties of surface and aerial petioles of the aquatic plant *Nymphaea odorata* subsp. *tuberosa* (*Nymphaeaceae*). *American Journal of Botany* **94**, 1067–1072.

**Evert RF.** 2006. *Esau’s plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development*: Hoboken, New Jersey (US): John Wiley & Sons.

**Floater G.** 1997. Rainfall, nitrogen and host plant condition: consequences for the processsionary caterpillar, *Ochrogaster lunifer*. *Ecological Entomology* **22**, 247–255.

**Garnier E, Laurent G.** 1994. Leaf anatomy, specific mass and water-content in congeneric annual and perennial grass species. *New Phytologist* **128**, 725–736.

**Garnier E, Salager JL, Laurent G, Sonie L.** 1999. Relationships between photosynthesis, nitrogen and leaf structure in 14 grass species and their dependence on the basis of expression. *New Phytologist* **143**, 119–129.

**Grassein F, Till–Bottraud I, Lavorel S.** 2010. Plant resource-use strategies: the importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. *Annals of Botany* **106**, 637–645.

**Hasan S, Shimojo M, Goto I.** 1993. Chemical components influencing lodging resistance of rice plant and its straw dideistibility in vitro. *Asian–Australasian Journal of Animal Sciences* **6**, 41–44.

**Hosden MJ, White PJ, Mead A, Broadley MR.** 2005. Phylogenetic variation in the silicon composition of plants. *Annals of Botany* **96**, 1027–1046.

**Hussner A, Meyer C, Busch J.** 2009. The influence of water level and nutrient availability on growth and root system development of *Myriophyllum aquaticum*. *Weed Research* **49**, 73–80.

**Jampetong A, Brix H.** 2009. Oxygen stress in *Salvinia natans*: interactive effects of oxygen availability and nitrogen source. *Environmental and Experimental Botany* **66**, 153–159.

**Kerpel SM, Soprano EO, Moreira GRP.** 2006. Effect of nitrogen on *Passiflora suberosa* L. (*Passifloraceae*) and consequences for larval performance and oviposition in *Heliconius erato phyllis* (Fabricius) (*Lepidoptera: Nymphalidae*). *Neotropical Entomology* **35**, 192–200.

**Koehl MAR.** 1982. The interaction of moving water and sessile organisms. *Scientific American* **247**, 124–132.

**Kokubo A, Kuraishi S, Sakurai N.** 1989. Culm strength of barley: correlation among maximum bending stress, cell wall dimensions, and cellulose content. *Plant Physiology* **91**, 876–882.

**Mony C, Pujalson S, Bornette G.** 2011. Resprouting response of aquatic clonal plants to cutting may explain their resistance to spate flooding. *Folia Geobotanica* **46**, 155–164.

**Niklas KJ.** 1992. *Plant biomechanics: an engineering approach to plant form and function*: Chicago, Illinois, USA: University of Chicago Press.

**Niklas KJ.** 1993. Influence of tissue density-specific mechanical properties on the scaling of plant height. *Annals of Botany* **72**, 173–179.

**Niklas KJ.** 1995. Plant height and the properties of some herbaceous stems. *Annals of Botany* **75**, 133–142.

**Onoda Y, Schieving F, Anten NPR.** 2008. Effects of light and nutrient availability on leaf mechanical properties of *Plantago major*: a conceptual approach. *Annals of Botany* **101**, 727–736.

**Poorter H, De Jong R.** 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytologist* **143**, 163–176.

**Pujalson S, Bouma TJ, Douady CJ, van Groenendael J, Anten NPR, Martel E, Bornette G.** 2011. Plant resistance to mechanical stress: evidence of an avoidance-tolerance trade-off. *New Phytologist* **191**, 1141–1149.

**Pujalson S, Lena J–P, Bornette G.** 2007. Interactive effects of nutrient and mechanical stresses on plant morphology. *Annals of Botany* **100**, 1297–1305.

**Pujalson S, Piola F, Bornette G.** 2008. Abiotic stresses increase plant regeneration ability. *Evolutionary Ecology* **22**, 493–506.

**R-Development-Core-Team.** 2009. R: a language and environment for statistical computing. *http://www.r-project.org*.
Nutrient enrichment affects plant strength

Rascio N. 2002. The underwater life of secondarily aquatic plants: some problems and solutions. Critical Reviews in Plant Sciences 21, 401–427.

Raven JA. 1996. Into the voids: the distribution, function, development and maintenance of gas spaces in plants. Annals of Botany 78, 137–142.

Raven JA. 2003. Cycling silicon: the role of accumulation in plants—commentary. New Phytologist 158, 419–421.

Ryser P. 1996. The importance of tissue density for growth and life span of leaves and roots: A comparison of five ecologically contrasting grasses. Functional Ecology 10, 717–723.

Ryser P, Gill HK, Byrne CJ. 2011. Constraints of root response to waterlogging in Alisma triviale. Plant and Soil 343, 247–260.

Sand–Jensen K. 2003. Drag and reconfiguration of freshwater macrophytes. Freshwater Biology 48, 271–283.

Sanson GD, Kerr SA, Gross KA. 2007. Do silica phytoliths really wear mammalian teeth? Journal of Archaeological Science 34, 526–531.

Santamaria L. 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. Acta Oecologica 23, 137–154.

Scholynck J, Bal K, Backx H, Okruszko T, Meire P, Struyf E. 2010. Silica uptake in aquatic and wetland macrophytes: a strategic choice between silica, lignin and cellulose? New Phytologist 186, 385–391.

Schulgasser K, Witzum A. 1997. On the strength of herbaceous vascular plant stems. Annals of Botany 80, 35–44.

Schutten J, Dainty J, Davy AJ. 2005. Root anchorage and its significance for submerged plants in shallow lakes. Journal of Ecology 93, 556–571.

Sculthorpe CD. 1967. The biology of aquatic vascular plants. London: Edward Arnold.

Shipley B, Vu TT. 2002. Dry matter content as a measure of dry matter concentration in plants and their parts. New Phytologist 153, 359–364.

Sorrell BK, Dromgoole Fl. 1988. Oxygen transport in the submerged freshwater macrophyte Egeria densa Planch. II. Role of lacunar gas pressures. Aquatic Botany 31, 93–106.

Sorrell BK, Tanner CC, Sukias JPS. 2002. Effects of water depth and substrate on growth and morphology of Eleocharis sphacelata: implications for culm support and internal gas transport. Aquatic Botany 73, 93–106.

Striker GG, Insausti P, Grimoldi AA, Vega AS. 2007. Trade-off between root porosity and mechanical strength in species with different types of aerenchyma. Plant, Cell and Environment 30, 580–589.

Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5, 537–542.

Usherwood JR, Ennos AR, Ball DJ. 1997. Mechanical and anatomical adaptations in terrestrial and aquatic buttercups to their respective environments. Journal of Experimental Botany 48, 1469–1475.

Valladares F, Gianoli E, Gómez JM. 2007. Ecological limits to plant phenotypic plasticity. New Phytologist 176, 749–763.

Van Arendonk JJCM, Niemann GJ, Boon JJ, Lambers H. 1997. Effects of nitrogen supply on the anatomy and chemical composition of leaves of four grass species belonging to the genus Poa, as determined by image-processing analysis and pyrolysis–mass spectrometry. Plant, Cell and Environment 20, 881–897.

Vogel S. 1984. Drag and flexibility in sessile organisms. American Zoologist 24, 37–44.

Vogel S. 1994. Life in moving fluids: the physical biology of flow. Princeton, New Jersey, USA: Princeton University Press.

Vogel S. 2003. Comparative biomechanics: life’s physical world: Princeton, New Jersey, USA: Princeton University Press.

Wallace A. 1989. Relationships among nitrogen, silicon, and heavy metal uptake by plants. Soil Science 147, 457–460.