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RESEARCH PAPER

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 witho 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; Schulgasser and Witztum, 1997).

In ecosystems 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 (Schoelynck *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)

and assigned to one nutrient condition (low or high nutrient level). 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 (Puijalon *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-P_2O_5 at high nutrient conditions compared with low nutrient ones. Four days after application of the treatment, concentrations of P-PO^{3-}_4, N-NO^-_3, and N-NH^+_4 were measured by the colorimetric method using a spectrometer (Easychem Plus SysteaTM). The concentrations were 24.35 versus 128.47 µg l^{-1} for [P-PO^{3-}_4], 1.94 versus 1.94 mg l^{-1} for [N-NO^-_3], and 4.09 versus 21.84 µg l^{-1} for [N-NH^+_4], 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 (mm^2)
(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 (mm^2)

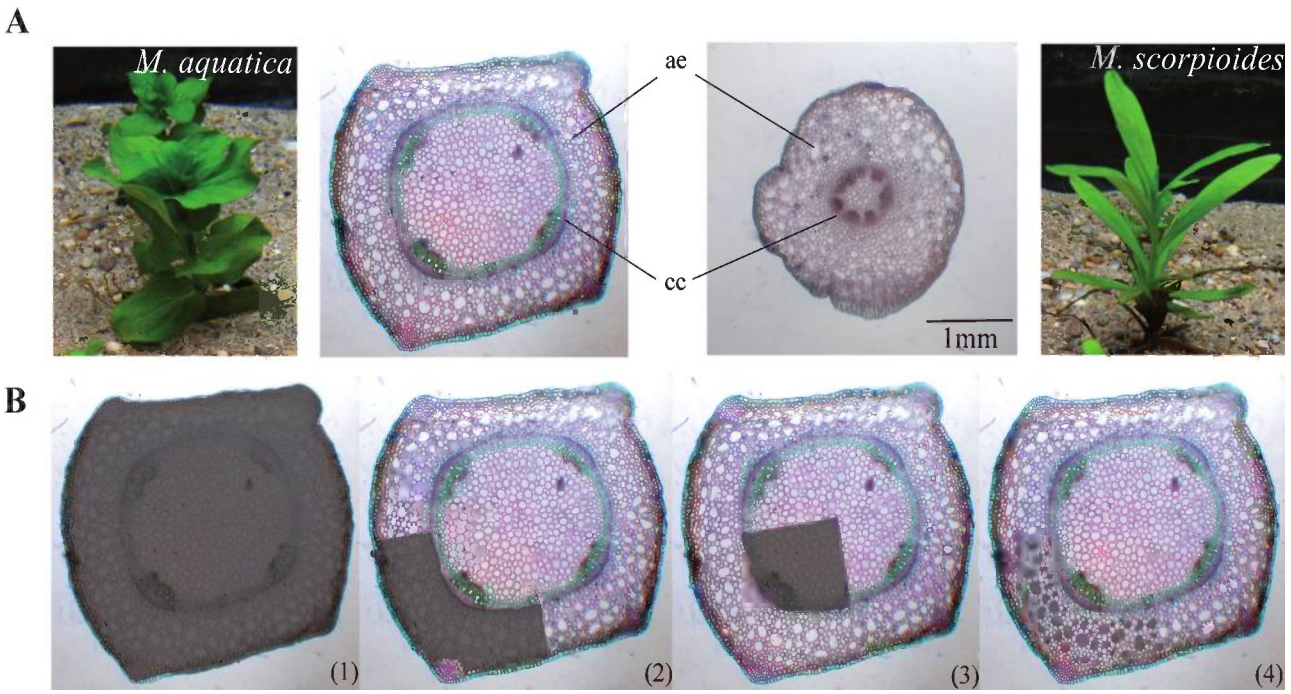

Fig. 1. (A) Entire plant and stem cross-section, showing aerenchyma (ae) and central cylinder (cc) position for the two species and (B) anatomical traits measured; (1) cross-sectional area, (2) aerenchyma area, (3) central cylinder area, and (4) lacunar area. (This figure is available in colour at *JXB* 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, 2003). 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 y of stem deformation 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 (m^4), 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* ($I_{myo}=\pi R^4/4$; *R: radius)* and a square cross-section for *M. aquatica* ($I_{men}=l^4/12$; *l: 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))* calculated as the ratio between the total lacunar area and the cross-sectional area (Choi *et al.*, 2007). 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).

$$\sigma = My/I \quad (1)$$

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

$$E=FL^3/192dI;\ d:\ deflection \quad (2)$$

(v) Flexural stiffness, EI ($N\ m^2$) 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 $\log_e$ 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 microscopic 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
	Dependent variable	**Log dry mass**	**Log dry mass**	**Log central cylinder area**	**Log total lacunar area**
	Covariate	**Log fresh mass**	**Log Volume**	**Log cross-sectional area**	**Log aerenchyma area**
M. aquatica	Covariate (C)	$F_{1.87}$=642.7 ***	$F_{1.70}$=100.4 ***	$F_{1.77}$=604.8 ***	$F_{1.78}$=42.66 ***
	Nutrient level (T)	$F_{1.86}$=3.77 ns	$F_{1.70}$=13.3 ***	$F_{1.77}$=5.79 *	$F_{1.77}$=0.19 ns
	C×T	$F_{1.85}$=0.38 ns	$F_{1.69}$=2.69 ns	$F_{1.76}$=1.56 ns	$F_{1.76}$=2.97 ns
M. scorpioides	Covariate (C)	$F_{1.58}$=252.6 ***	$F_{1.37}$=50.53 ***	$F_{1.48}$=68.1 ***	$F_{1.48}$=281.9 ***
	Nutrient level (T)	$F_{1.58}$=7.32 **	$F_{1.37}$=4.24 *	$F_{1.47}$=0.34 ns	$F_{1.47}$=0.1 ns
	C×T	$F_{1.57}$=2.17 ns	$F_{1.36}$=2.54 ns	$F_{1.46}$=0.32 ns	$F_{1.46}$=2.02 ns

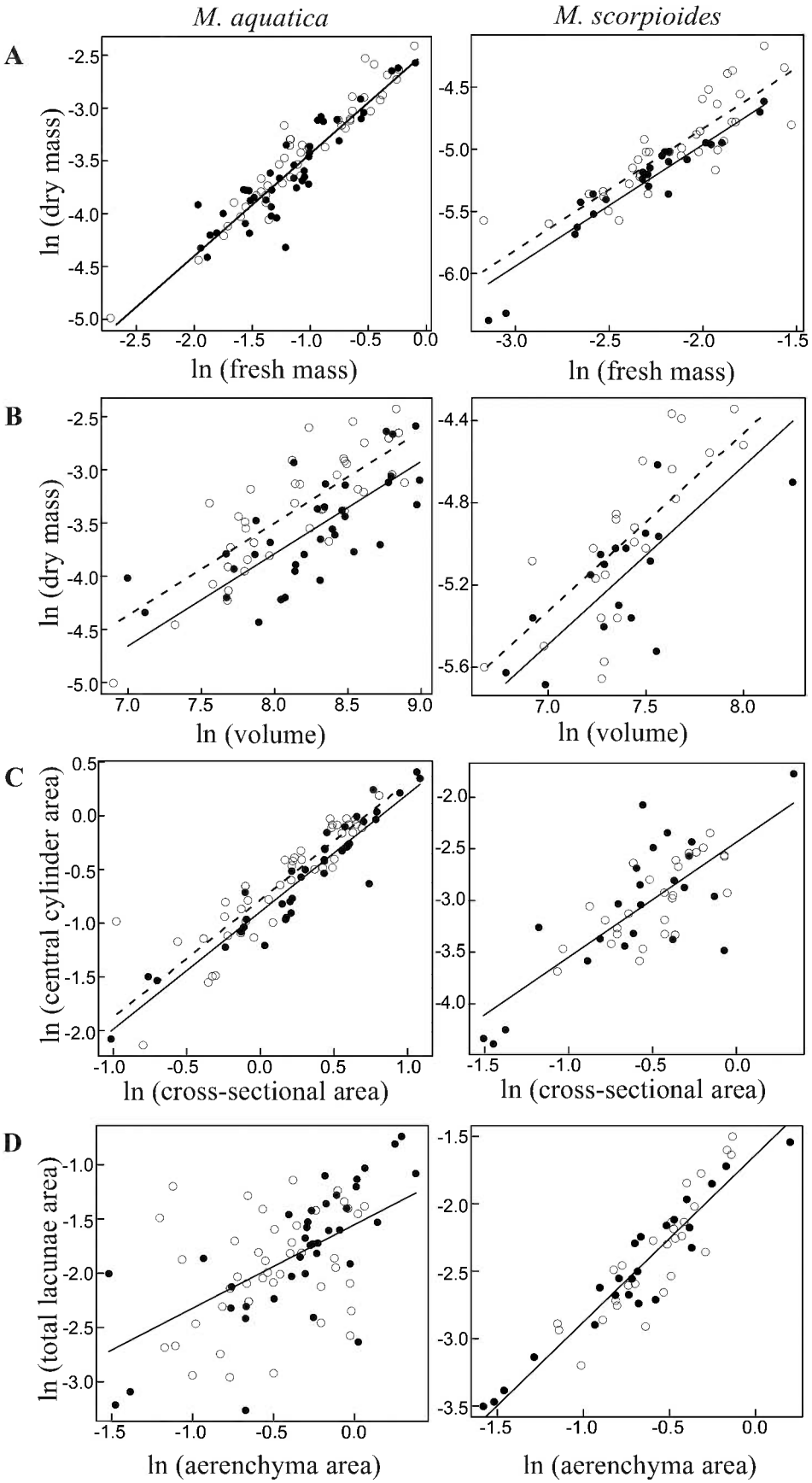

Fig. 2. Effects of nutrient level on (A) DMC, (B) D, (C) central cylinder area relative to cross-sectional area, and (D) total lacunar area relative to aerenchyma area for *M. aquatica* and *M. scorpioides*. Points represent values and line regressions of ANCOVA; (open circles) low nutrient level; (filled circles) high nutrient level.

(Table 1, Fig. 2D), but average individual lacunar area was significantly higher under high nutrient conditions ($t_{72.4}$= –2.56, P=0.01). For *M. scorpioides,* neither total lacunar area relative to aerenchyma area (Table 1; Fig. 2D) nor average individual lacunar area differed between nutrient conditions ($t_{32.3}$= –0.54).

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 (Schulgasser and Witztum, 1997; Usherwood *et al.*, 1997; Etnier 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 (I, second moment area; *Icor*, second moment area corrected by porosity; deflection; breaking force; σ, bending stress; σcor: breaking stress corrected for porosity, E, elastic modulus; *Ecor*, elastic modulus corrected for porosity; EI, flexural stiffness).

	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 ddor Welch test**
I (mm^4)	0.57 ± 0.27	0.47 ± 0.18	ns (t)	2.68 ± 1.95	3.40 ± 2.30	ns (t)
Icor (mm^4)	0.47 ± 0.18	0.39 ± 0.15	ns (t)	2.39 ± 1.63	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)
σ (MPa)	19.39 ± 9.91	8.09 ± 10.97	** (w)	16.96 ± 7.55	12.06 ± 12.48	ns (t)
σcor (MPa)	22.88 ± 13.92	9.48 ± 5.91	*** (w)	20.54 ± 12.57	13.65 ± 13.41	* (t)
E (MPa)	55.66 ± 30.85	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)
EI (10^{-5}N m^2)	2.58 ± 1.01	1.02 ± 0.93	*** (w)	8.27 ± 2.88	6.56 ± 3	* (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. Silica, 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 (H_4SiO_4) and polymerized into a crystallized form ($SiO_2.nH_2O$) 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. NH^+_4) to anion uptake may induce a proton excretion, acidifying the environment, which makes silica less soluble (Wallace, 1989). As silica 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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