

A comprehensive analysis of mechanical and morphological traits in temperate and tropical seagrass species

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ABSTRACT: Knowledge of plant mechanical traits is important in understanding how plants resist abiotic and biotic forces and in explaining ecological strategies such as leaf lifespan. To date, these traits have not been systematically evaluated in seagrasses. We analysed mechanical (breaking force and tensile strength) and associated traits (thickness, width, length, fibre content, mass area, and lifespan) of leaves in 22 seagrass species (around one-third of all known seagrass species) to examine (1) the inter-specific variation of these traits in relation to growth form and bioregions, (2) the contribution of morphology to leaf breaking force, (3) how breaking force scales to leaf dimensions, and (4) how mechanical and structural traits correlate to leaf longevity. We also compared our seagrass dataset with terrestrial plant databases to examine similarities between them. Large variation in leaf breaking force was found among seagrass species but, on average, temperate species resisted higher forces than tropical species. Variation in leaf breaking force was largely explained by differences in leaf width rather than thickness, likely due to the benefits in leaf reconfiguration and light interception. Species of large dimensions (long leaves) typically had high leaf breaking force, plausibly to tolerate the drag forces they may experience, which are proportional to the leaf area. Leaves of long-lived species typically had high mass per leaf area and fibre content and they supported high breaking forces. Compared to terrestrial plants, seagrasses are short-lived species with moderately strong fibre-reinforced leaves, which probably evolved to withstand the hydrodynamic forces occurring in the sea, and in response to other environmental factors. Overall, our analysis provides new insights into the physical performance of seagrasses in the marine environment.

KEY WORDS: Biomechanics · Seagrass · Breaking force · Leaf mass per area · Leaf size · Fibre content · Leaf lifespan

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INTRODUCTION

Knowledge of plant mechanical traits is important to understand how plants resist abiotic (e.g. fluid-driven forces) and biotic (herbivory) mechanical forces (Read & Stokes 2006). Plant biomechanics

have been relatively well studied in land plants from local to global scales (Onoda et al. 2011), and are important to plant ecological strategies such as leaf lifespan and also to ecosystem processes such as litter decomposition, plant–herbivore interactions and nutrient cycling (Read & Stokes 2006). Studies on

mechanical properties of aquatic plants are far fewer (e.g. Puijalon et al. 2011, Miler et al. 2014) and most studies on marine macrophytes are mainly concentrated on macroalgae (e.g. Koehl 1984, Duggins et al. 2003, Harder et al. 2006) whereas only a few studies have examined the biomechanics of seagrasses (Kopp, 1999, Patterson et al. 2001, de los Santos et al. 2012, 2013, La Nafie et al. 2012, 2013), contrasting with the ecological relevance of this group of foundation species (Duffy 2006).

Seagrasses are unique angiosperms, representing 3 or 4 separate land plant lineages which have evolved to grow in marine habitats (Den Hartog 1970, Waycott et al. 2006), making them ideal model plants to study the mechanical traits required to live in marine environments. About 60 seagrasses are known and widely distributed along temperate and tropical coasts of the world (Den Hartog 1970, Short et al. 2007), where they form extensive meadows in a wide variety of habitats, from sheltered water bodies to coastal areas exposed to strong currents, and even in surf zones (Koch et al. 2006). Thus, many seagrasses face strong physical forces due to water motion, which pose a risk of physical damage such as breakage or dislodgment (Koch et al. 2006). Drag force due to water movement is one of the main forces experienced by aquatic plants, and it is proportional to the product of their frontal area, the flow velocity, and the density of the fluid (Niklas 1992, Vogel 1994). As water is 800 times denser than air, the drag force imposed on aquatic plants is often much higher than forces exerted by wind on terrestrial plants (Niklas 1992). To resist and survive such drag forces, aquatic plants have either a reduced size or flexible, streamlined leaves to reconfigure with the flow (avoidance strategy, e.g. Ennos 1999, Sand-Jensen 2003), or they are tough enough to tolerate the drag forces (tolerance strategy, e.g. Bouma et al. 2005, Puijalon et al. 2011). Despite their independent lineages, most seagrass species have converged to a form of flexible, narrow, unbranched leaves arising from the rhizome, but with a considerable range of leaf lengths (Duarte 1991), from a few centimetres (*Halophila* spp.) to several meters (*Zostera caulescens*; Aioi et al. 1998). In addition to this major group, there are also species with oval leaves arising from petioles (i.e. genus *Halophila*) or terete leaves (genera *Syringodium* and *Phyllospadix* and a species in the *Posidonia ostenfeldii* 'complex') and a group of species with a cluster of strap-like leaves arising from vertical stems (e.g. genera *Amphibolis* and *Thalassodendron*) (Den Hartog 1967). However, the physical tolerance limits of seagrasses have not been system-

atically and quantitatively evaluated to date, with only some studies focussed on single or small groups of species (Patterson et al. 2001, de los Santos et al. 2012, 2013, La Nafie et al. 2012, 2013).

The maximum capacity of a plant structure (leaf, petiole or stem) to withstand a force (termed 'breaking force' hereafter) is the major mechanical property contributing to their tolerance strategy (Puijalon et al. 2011). Breaking force is the product of 2 components—cross-sectional area and tensile strength (Niklas 1992, Vogel 1994). Tensile strength is an intrinsic property of any material, and in plant leaves it is tightly associated with the amount and arrangement of fibres (e.g. Onoda et al. 2011). Thus, to tolerate drag forces, plants should have either large cross-section areas (thickness or width), high tensile strength, or both. However, these features come at some costs. Leaf thickening constrains the leaf's ability to bend and reconfigure with water movement, which in turn increases the drag force, because flexural stiffness increases by a power of 3 with thickness (Niklas 1992). Leaf widening increases the leaf frontal area and may consequently increase the drag force (e.g. Bouma et al. 2005). Leaf strengthening also requires more fibres and thus increases construction costs per unit volume. Each of these 3 ways to increase tolerance to the hydrodynamic forces incurs different costs and benefits, and clarifying how seagrasses cope gives insights about how they have diversified to various environmental conditions.

In terrestrial plants, leaf toughening is associated with leaf mass per area, a key functional trait reflecting the leaf economics strategy (Wright et al. 2004). According to this concept, high leaf mass per area leaves tend to be more structurally resistant, which may also be beneficial for protection against herbivory and other physical damage, and consequently to achieve long leaf lifespans (Reich et al. 1991, Wright et al. 2004, Read & Stokes 2006). Seagrasses present a high variability in leaf longevity, from a few days in *Halophila ovalis* to almost a year in *Posidonia oceanica* (Duarte 1991), which has been related to leaf weight and bioregions, where temperate species or heavier leaves tend to live longer than tropical species or more light-weight leaves (Hemminga et al. 1999). However, it remains unclear whether leaf mechanical resistance and structural components are associated with leaf longevity across seagrass species, as observed for terrestrial plants (Wright et al. 2004, Onoda et al. 2011) and, if so, whether the trait relationships in seagrasses follow the same patterns as in terrestrial plant species.

In this study, we aimed at providing for the first time a comprehensive analysis of the mechanical and associated traits of seagrasses to understand how these unique marine plants face the particular hydrodynamic environment of the sea and how it correlates to their leaf lifespan. We analysed mechanical, morphological and structural traits of 22 seagrass species collected from temperate and tropical regions and from contrasting environments, accounting for one-third of seagrass species on Earth. These seagrasses cover the 3 major growth forms of seagrasses and a wide spectrum of sizes and leaf lifespans. We specifically examined the following questions:

(1) How do mechanical traits vary among species, among growth-forms, and between temperate and tropical bioregions?

(2) How does breaking force scale to leaf size traits?

(3) To what extent do size traits (thickness, width) and tensile strength contribute to variation in the leaf breaking resistance across and within species?

(4) Do breaking force, leaf mass per area, and fibre content explain the variation in leaf lifespan across seagrass species? And if so, is the pattern different from that in terrestrial plants?

MATERIALS AND METHODS

Plant collection and sampling sites

We collected 22 seagrass species covering all families (Hydrocharitaceae, Posidoniaceae, Cymodoceae, Zosteraceae, Ruppiaceae) and 13 out of the 14 genera (only *Phyllospadix* was absent; Table S1 in Supplement 2 at www.int-res.com/articles/suppl/m551/p081_supp.pdf), representing ca. one-third of existing seagrass species. The collected species covered the 3 major growth-forms of seagrasses (Den Hartog 1967): (G1) plants with strap-like leaves arising directly from the rhizome (16 species: *Cymodocea nodosa*, *C. rotundata*, *C. serrulata*, *Enhalus acoroides*, *Halodule pinifolia*, *H. uninervis*, *Posidonia australis*, *P. coriacea*, *P. oceanica*, *P. sinuosa*, *Ruppia maritima*, *Syringodium isoetifolium*, *Thalassia hemprichii*, *Zostera capricorni*, *Z. marina*, and *Z. noltei*); (G2) plants with strap-like leaves arising from a vertical stem (4 species: *Amphibolis antarctica*, *A. griffithii*, *Thalassodendron pachyrhizum*, and *Z. nigricaulis*); and (G3) plants with oval leaves in pairs attached to the rhizome through a petiole (2 species: *Halophila ovalis* and *H. ovalis* ssp. *bullosa*). The specimens were sampled between October 2007 and

September 2010 at 19 sites (Table S1) in 3 out of the 6 world seagrass bioregions (Short et al. 2007): 1 tropical (Tropical Indo-Pacific bioregion), and 2 temperate (Mediterranean–Atlantic and Temperate Southern Oceans bioregions). The Tropical Indo-Pacific bioregion accounts for the greatest seagrass diversity, with mixed meadows, predominantly on reef flats and lagoons and grazed by mega-herbivores. The Mediterranean–Atlantic bioregion is characterised by vast meadows bearing moderate biodiversity of seagrasses, which are normally found in sheltered areas, but also may be semi-exposed to waves and currents. The Temperate Southern Oceans bioregion includes extensive meadows with a diverse range of number of species, often growing under extreme hydrodynamic conditions, i.e. in coastal areas exposed to open ocean with a strong wave action (Caruthers et al. 2007, Short et al. 2007). Depending on logistic feasibility, individuals of the same species were collected from populations with different habitat conditions and/or at different times of the year (Table S1), in order to capture their phenotypic variation in mechanical traits (de los Santos et al. 2013). Some species, however, were only sampled from 1 site and may not reflect all the intra-specific variation; nevertheless, this study was mainly focussed on interspecific variation. The final database accounted for 55 species-at-site sets divided into 8 species and 21 species-at-site sets from the Tropical Indo-Pacific bioregion (ca. one-third of existing species in that bioregion), 5 species and 12 species-at-site sets from the Mediterranean–Atlantic bioregion (full representation of endemic seagrasses), and 10 species and 22 species-at-site sets from the Temperate Southern Oceans bioregion (ca. half of existing species from that bioregion) (Table S1). At each site, 5–15 entire shoots (leaf bundle with rhizome and roots) were randomly collected in intertidal or subtidal meadows by snorkelling or scuba diving. Plants were carefully handled to minimise physical damage and loss of water content: they were horizontally laid, totally wrapped in moist tissue, placed in plastic bags completely sealed to avoid desiccation, then transported to the laboratory under cool dark conditions. Fijian, Indonesian, and Western Australian species were transported by plane to the laboratory where the measurements were carried out. After arrival (less than 48 h from collection), healthy plants were immediately submerged in tanks with aerated natural seawater at 20°C (temperate species) or 29°C (tropical species) in a climate-controlled chamber until tested. This time of storage does not alter the mechanical properties of seagrasses (de los Santos et al. 2016).

Mechanical traits

Mechanical properties were measured on the leaf blade and sheath in growth-form G1 (fragments of ca. 4–5 cm above and below their junction, respectively), leaf blade and stem (central part) in G2, and leaf and petiole in G3. Fully developed, non-senescent, healthy leaves of similar age (given by the leaf rank position within the shoot for each species) and without herbivory marks were selected for all measurements. Epiphytes, if present, were carefully removed from the leaf surface with paper tissue. Selected leaves in some species did not present a fully developed sheath, so only leaf blades were tested in those cases. From 5 to 15 replicates were used for each species-at-site (Table S1), following standardised protocols in plant trait analyses (Pérez-Harguindeguy et al. 2013). Final dataset resulted in 1143 valid measurements, distributed among 744 leaf blades, 336 sheaths, 24 stems, and 39 petioles.

Mechanical properties were obtained from tensile tests conducted with a tensometer (models 5542 or 3340, Instron® and BlueHill® software v. 2.18; High Wycombe) at the University of Utrecht (The Netherlands), Macquarie University (Australia), and University of Cádiz (Spain), using a 5-N load cell for smaller species and a 500-N load cells for bigger species, and 5- or 250-N pneumatic action grips (model 2712, Instron®). Selection of the load cell and grips was based on preliminary tests for each species. Samples were tested within 24 to 72 h after collection, in sequence by replicates, not by species, so that the time of storage was homogeneous across them. For all measurements, plant fragments were excised immediately before being tested and kept moist with seawater all the time. Specimen dimensions (length, thickness, and width or diameter, and one-side area) were measured before testing, whereas dry mass was measured after testing (see next subsection). Tests were conducted in parallel to the main axis of specimens, including the leaf midrib in *Halophila* spp. and *Halodule* spp. Specimens were individually clamped into the grips with the gauge length set to 20 mm. They were stretched at a constant velocity of 10 mm min⁻¹, while the displacement (δ , mm) and the force (F, N) were recorded every 0.1 second until mechanical failure occurred, recording the breaking force (N) and the increase in length (mm) at the breaking point. Tensile strength (N mm⁻²) was calculated as the breaking force per unit of initial cross-section area (mm²). Extensibility (%) was determined as increase in length at the breaking point relative to the original length. Stiffness (N mm⁻²) was given by

the Young's modulus of elasticity, which is calculated from the initial slope of the force-displacement curve (F/δ), the initial length (mm), and the cross-sectional area (mm²) of the specimen. Each test was carefully monitored to exclude those specimens that slipped during the test or broke in or close to the grips.

Non-mechanical traits

Leaf size (length, thickness, and width), leaf mass per area, and chemically extracted fibre content were measured in the same fragments used in the tensile tests (but fibre content, leaf length, and leaf mass per area were not determined in Fijian species). Leaf length was determined with a ruler (± 0.1 cm), leaf width with a digital calliper (± 0.01 mm), and leaf thickness or diameter(s) with a thickness gauge (± 0.01 mm). Leaf thickness was measured avoiding midribs (*Halophila* spp., *Halodule* spp.) or side fibres (*Enhalus acoroides*). Leaf cross-section area (mm²) was calculated according to geometrical approximation (round cross-section area considered for leaves of *Syringodium isoetifolium* and petioles of *Halophila* spp., elliptical cross-section area for stems, and rectangular for the rest). One-sided leaf area (cm²) was measured in fresh leaves by image analysis (ImageJ, US National Institutes of Health; www.nih.gov/) after being scanned in a flatbed scan. Leaf mass per area (g m⁻²) was then determined as the dry mass divided by leaf frontal area. Fibre content (% dry mass) was measured in pulverised dry biomass following the Van Soest method (Van Soest et al. 1991) with minor modifications (described in de los Santos et al. 2013). Briefly, this procedure gives the neutral detergent fibre content as the difference in dry mass after digestion. Samples of dry biomass (20–30 mg) were heated to boiling (100°C) in 2 ml of neutral detergent (Ankom FND20C) for 1 h, followed by repetitive washings of the pellet (2500 $\times g$, 5 min) with 2 ml of distilled water ($\times 2$), ethanol ($\times 2$), and acetone ($\times 1$), and finally being oven-dried (60°C, 24 h). In seagrasses, fibre content roughly represents the amount of cellulose, hemicellulose, and, when present, lignin (Kuo & Den Hartog 2006).

Leaf lifespan (d) was compiled from literature for 18 species (values absent for: *H. ovalis* spp. *bullosa*, *Z. nigricaulis*, *R. maritima*, and *H. pinifolia*; Table S2 in Supplement 2). Values were obtained either directly from the sources, calculated as the mean number of standing leaves divided by the number of leaves produced per shoot during a year, or as the inverse of the annual leaf turnover, as in previous

compilations (Duarte 1991, Hemminga et al. 1999). Average leaf lifespan was calculated when more than one source was available for a species. When values were given in a maximum-minimum range, the mean of the range was used. For many species, the compiled values corresponded to populations from different sites to those from where our samples were collected. Nevertheless, given that the coefficient of variation of leaf lifespan within-species (21%) is much lower than among-species (113%; Duarte 1991), we assumed that the attribution of compiled values to our data was sufficient for across-species correlations.

Comparison to terrestrial plants

We used 2 global databases to retrieve average values of leaf traits of terrestrial plant species (Wright et al. 2004, Onoda et al. 2011). We compared them to our dataset of seagrass species to find similarity or dissimilarity between the 2 groups, in order to investigate what type of traits are required to survive in the marine environment as compared to the terrestrial environment. Furthermore, we highlighted herbaceous species data in the comparison because woody species and herbaceous species are very different in their traits (Díaz et al. 2016) and seagrasses are herbaceous plants.

Data and statistical analyses

Across-species comparisons were based on species-averaged data, independently of the site or time of collection, and presented as means \pm SD. The extent of variation of leaf traits across- and within-species was assessed by the coefficient of variation (%CV). Two different linear mixed-effects models were designed to test differences in leaf traits (\log_{10} -transformed data) among growth-forms (Model A) and bioregions (Model B). In Model A, growth-form was included as fixed factor and species nested to growth-form was included as random factor. In Model B, bioregion was included as fixed factor and species-at-site nested to bioregion as random factor. In both cases, models were fit by maximum likelihood and type III Wald χ^2 tests were used to assess the significance of the fixed effect in the model. When the effect of fixed factors was significant, homogenous groups were obtained from Tukey's multiple comparisons tests. Visual inspection of residual plots did not reveal any obvious deviation

from homocedasticity or normality in the linear models.

Influential points were explored prior to correlation and regression analyses using the Cook's distance criteria and, if observed, they were excluded from the analysis and an ecological explanation was given to account for their unusual values. Model II linear regression (standardised major axis, SMA; Warton et al. 2006) on species-averaged \log_{10} -transformed data were used to scale breaking force to leaf dimensions (surface area, width, and thickness) and tensile strength. SMA linear regression was used to prevent bias in regression coefficients derived from error in both variables (LaBarbera 1989). Since breaking force is calculated as the product of 3 components (thickness, width, and tensile strength), the contribution of each component was assessed by a quantitative analysis using variance partitioning of the components, as previously used in similar analysis (Onoda et al. 2011). Briefly, the variance of the breaking force is denoted as the sum of the covariances between each component and the breaking force. When the covariance of each component is taken as a proportion of the variance of the breaking force, the relative contribution of each component can be obtained (complete algorithms in Supplement 1 at www.int-res.com/articles/suppl/m551p081_supp.pdf). The contribution of each component to the breaking force was calculated for all species altogether (across-species) and separately for each species (within-species). Only species with at least 10 replicates were used in the within-species variance partitioning analysis.

Pearson's correlation coefficient was used to test the degree of association between species-averaged pairs of traits. In addition, the phylogenetic independent contrasts (PICs) method (Felsenstein 1985, Garland et al. 1992) was used to test whether trait correlations in seagrasses were due to a functional relationship or simply due to their shared phylogeny. A seagrass phylogenetic binary tree with species resolution was developed for the 22 species included in this study. Seagrass phylogenetic data were based on strict consensus in Les et al. (1997), using the associated newick file from TreeBASE repository (study S618, www.treebase.org). The phylogenetic binary tree was then adjusted according to Waycott et al. (2006) to include the rest of species in this study and their branch lengths. Phylogenetic data were not available for *Zostera nigricaulis*, *Amphibolis griffithii*, and *Halophila ovalis* spp. *bullosa*, so they were located as terminal nodes next to their congeneric species. Branch lengths were not available for all

Table 1. Summary of seagrass leaf traits for all species (overall) and grouped by growth-forms (G1: strap-like leaves from the rhizome; G2: strap-like leaves from a stem; G3: oval leaves with petioles). Data shown as mean \pm SD (median). CV: coefficient of variation among species. Lettering indicates homogeneous groups from Tukey's multiple comparison among growth-form groups when significant differences were found in the linear-mixed effects model (growth-form as a fixed factor and species nested to group as a random factor). LMM: Linear mixed-effects model. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Leaf trait	CV	Overall (n = 22)	G1 (n = 16)	G2 (n = 4)	G3 (n = 2)	LMM χ^2
Mechanical						
Breaking force (N)	108	10.46 \pm 11.31 (5.7)	11.3 \pm 12.82 (5.46)	10.49 \pm 5.99 (10.45)	3.66 \pm 3.15 (3.66)	1.4
Tensile strength (N mm ⁻²)	61	5.43 \pm 3.29 (4.97)	5.02 \pm 3.64 (4.65)	7.62 \pm 1.27 (7.12)	4.32 \pm 0.66 (4.32)	2.9
Extensibility (%)	53	8.16 \pm 4.29 (6.84)	6.83 \pm 3.64 (5.4) ^a	10.6 \pm 3.89 (11.93) ^{ab}	13.89 \pm 5.03 (13.89) ^b	9.3**
Stiffness (N mm ⁻²)	78	104.59 \pm 81.27 (79)	114.22 \pm 90.77 (98.5)	92.73 \pm 46.52 (72.12)	51.3 \pm 30.05 (51.3)	1.1
Non-mechanical						
Mass per area (g m ⁻²)	46	55.76 \pm 25.71 (45.5)	50.9 \pm 13.84 (49.38) ^{ab}	69.51 \pm 30.7 (65.08) ^a	26.6 \pm 9.25 (28.84) ^{b,1}	6.2*
Fibre content (% DW)	15	47.71 \pm 7.18 (47.3)	47.58 \pm 7.99 (46.77)	48.98 \pm 4.86 (48.61)	44.65 \pm 4.84 (44.27) ¹	0.36
Length (cm)	82	25.82 \pm 21.12 (15.97)	30.49 \pm 21.73 (18.82) ^a	13.7 \pm 11.37 (10.47) ^{ab}	3.3 \pm 0.37 (3.3) ^{b,1}	9.5**
Frontal area (cm ²)	120	18.59 \pm 22.33 (7.48)	21.45 \pm 23.82 (10.11)	10.98 \pm 16.4 (2.91)	4.01 \pm 0.83 (3.92) ¹	0.7
Width (mm)	68	6.66 \pm 4.54 (6.62)	5.83 \pm 4.38 (4.69)	7.92 \pm 4.94 (7.64)	10.71 \pm 4.74 (10.71)	2.7
Thickness (mm)	103	0.36 \pm 0.37 (0.23)	0.43 \pm 0.41 (0.29) ^a	0.20 \pm 0.04 (0.18) ^{ab}	0.07 \pm 0.03 (0.07) ^b	13.9***
Cross-section area (mm ²)	112	2.29 \pm 2.56 (1.54)	2.67 \pm 2.9 (1.54)	1.55 \pm 0.86 (1.73)	0.81 \pm 0.59 (0.81)	0.55

¹Values exclusively for *Halophila ovalis* due to absence of data for *H. ovalis* ssp. *bullosa*

nodes so a default value of 100 was attributed when absent. A critical α level of 0.05 was used for all hypothesis tested. All data and statistical analyses were done using R programming software (v3.0.3; R Development Core Team 2014).

RESULTS

Variation among species, growth-forms, tissues and bioregions

Leaf breaking force, cross-section area, and tensile strength varied respectively 50-, 85-, and 20-fold among species (Table 1, Table S3 in Supplement 2 at www.int-res.com/articles/suppl/m551p081_supp.pdf). *Posidonia* spp., *Enhalus acoroides*, and *Thalassodendron pachyrhizum* supported the highest forces (breaking force ca. 20–36 N), while *Zostera noltei*, *Ruppia maritima*, *Halodule pinifolia*, *Halophila ovalis* ssp. *bullosa* and *Syringodium isoetifolium* recorded the lowest (ca. 0.7–1.5 N), and the remaining species were generally below the overall mean (10.2 N; Fig. 1a). Leaf cross-section area varied greatly among species (112% CV), showing a similar species rank for leaf breaking force (Fig. 1b). In contrast, tensile strength was less variable among species (61% CV; Fig. 1c), and the strongest tissues were recorded in leaves from *P. sinuosa*, *Amphibolis antarctica*, *A. griffithii*, *T. pachyrhizum*, *Z. nigricalis*, and *R. maritima*. Stiffness and extensibility

varied 23- and 5-fold across-species, respectively (Table S3). *P. sinuosa* and *P. oceanica* were very stiff in tension (260–360 N mm⁻²); and *Halophila* spp., *R. maritima*, *Amphibolis* spp., *T. pachyrhizum*, *P. coriacea*, and *Halophila* spp. presented a high extensibility (>10% increase in length, Table S3).

Due to a substantial variation in mechanical traits within growth-form, there were no significant differences among groups in these traits (Fig. 2, Table 1). The most significant differences of groups G2 and G3, in comparison to G1 (the common growth-form in seagrasses) were in non-foliar structures: stems of species in G2 were very strong (13.27 \pm 5.25 N mm⁻²), extensible (19.79 \pm 14.29%), and stiff (197.03 \pm 62.28 N mm⁻²), whereas petioles in species from G3 were weak (2.04 \pm 0.52 N mm⁻²), extensible (54.17 \pm 5.72%), and not very stiff (8.11 \pm 5.32 N mm⁻²) (Fig. 2). Regarding G1, both leaf and sheaths covered almost the whole range of values, although leaf sheaths were slightly weaker, less stiff, and more extensible than leaf blades, but both presented similar breaking forces (Fig. 2).

Seagrass leaves from Temperate Southern Oceans resisted ca. 2.3–3.7 times higher forces ($\chi^2 = 21.6$, $p < 0.001$) than leaves from the other 2 bioregions: Tropical Indo-Pacific and Mediterranean–Atlantic (Fig. 3). Tensile strength ($\chi^2 = 38.6$, $p < 0.001$) was 1.7–2.5 times higher in leaves from temperate seagrasses (Temperate Southern Oceans and Mediterranean–Atlantic bioregions), than in leaves from the Tropical Indo-Pacific bioregion (Fig. 3).

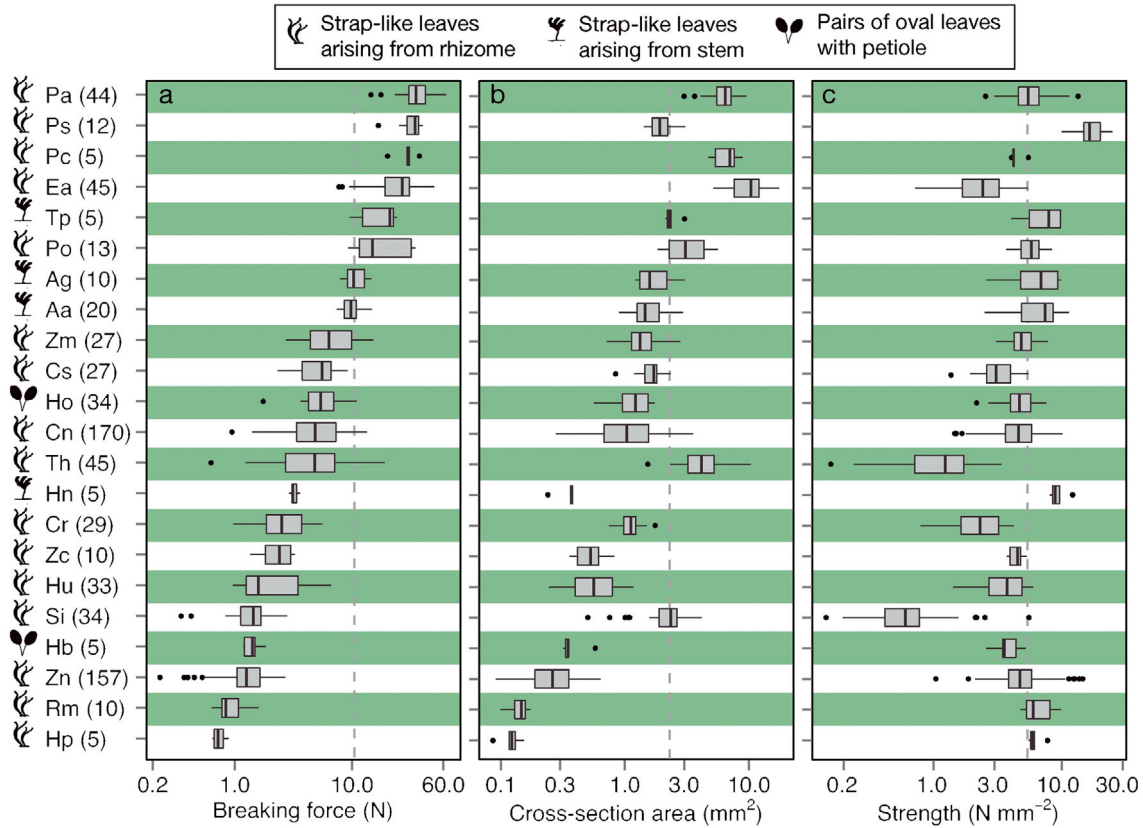


Fig. 1. Breaking force, cross-section area and tensile strength of seagrass leaves by species. Number of observations in parentheses. Symbols refer to growth-form group. Dashed line indicates mean value for all species. Boxplot bar: median; box: interquartile range (IQR); whiskers: max./min. values within $1.5 \times$ IQR of the hinge; dots: outliers. Species abbreviations: *Amphibolis antarctica* (Aa), *A. griffithii* (Ag), *Cymodocea nodosa* (Cn), *C. rotundata* (Cr), *C. serrulata* (Cs), *Enhalus acoroides* (Ea), *Halodule pinifolia* (Hp), *H. uninervis* (Hu), *Halophila ovalis* (Ho), *H. ovalis* ssp. *bullosa* (Hb), *Posidonia australis* (Pa), *P. coriacea* (Pc), *P. oceanica* (Po), *P. sinuosa* (Ps), *Ruppia maritima* (Rm), *Syringodium isoetifolium* (Si), *Thalassia hemprichii* (Th), *Thalassodendron pachyrhizum* (Tp), *Zostera capricorni* (Zc), *Z. marina* (Zm), *Z. nigricaulis* (Zg), and *Z. noltei* (Zn)

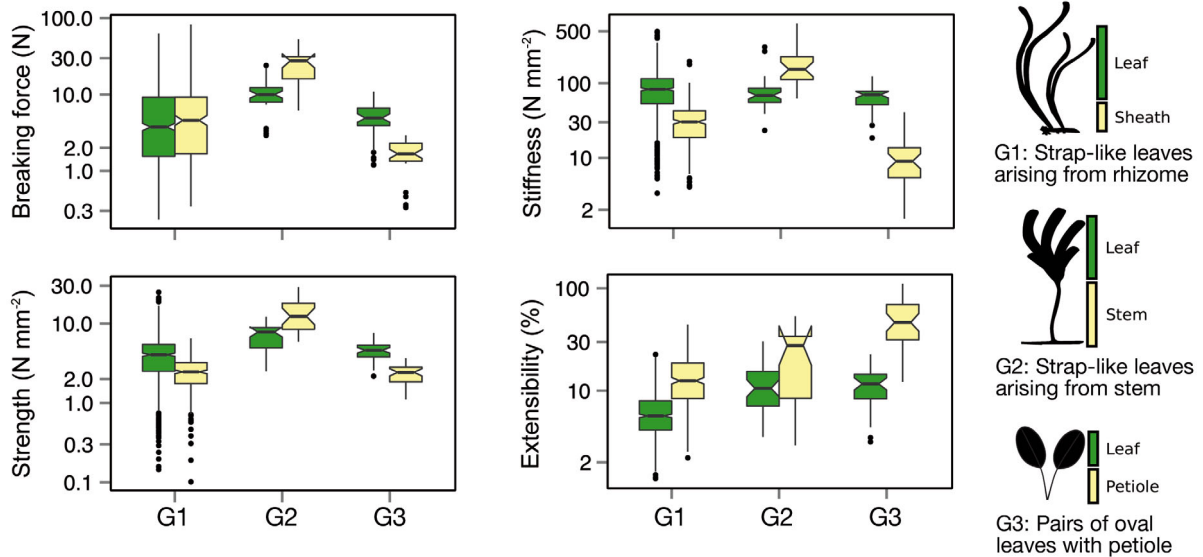


Fig. 2. Mechanical properties of plant parts (leaf, sheath, stem, and petiole) of seagrasses by growth-form (G1–G3). Boxplot bar: median; notch: 95% confidence interval around median; box: interquartile range (IQR); whiskers: max./min. values within $1.5 \times$ IQR of the hinge; dots: outliers. Notches are used to compare values among groups and plant parts: if the notches of 2 boxes do not overlap, this is strong evidence that the medians differ

Contribution of morphology to breaking force and scaling relationships

Leaf breaking force scaled to frontal area with a scaling slope less than 1 ($n = 18$, $p < 0.001$, $R^2 = 0.92$), when excluding the short-leaved species of the genus *Amphibolis* (Fig. 4a). This scaling relationship meant that the increase in breaking force was less than proportional to the increase in frontal area. On the contrary, the scaling slopes of breaking force with width ($n = 22$, $p = 0.033$, $R^2 = 0.62$) and thickness ($n = 22$, $p < 0.001$, $R^2 = 0.16$) were greater than 1 (Fig. 4b,c), reflecting additive effects of width and thickness on the breaking force. The scaling relationship with thickness was strengthened ($n = 21$, $p < 0.001$, $R^2 = 0.43$) after excluding the distinctive species *S. isoetifolium*, which has thick terete leaves (Fig. 4c). On the contrary, leaf strength did not scale significantly with breaking force among species ($n = 22$, $p = 0.107$, $R^2 = 0.08$, Fig. 4d).

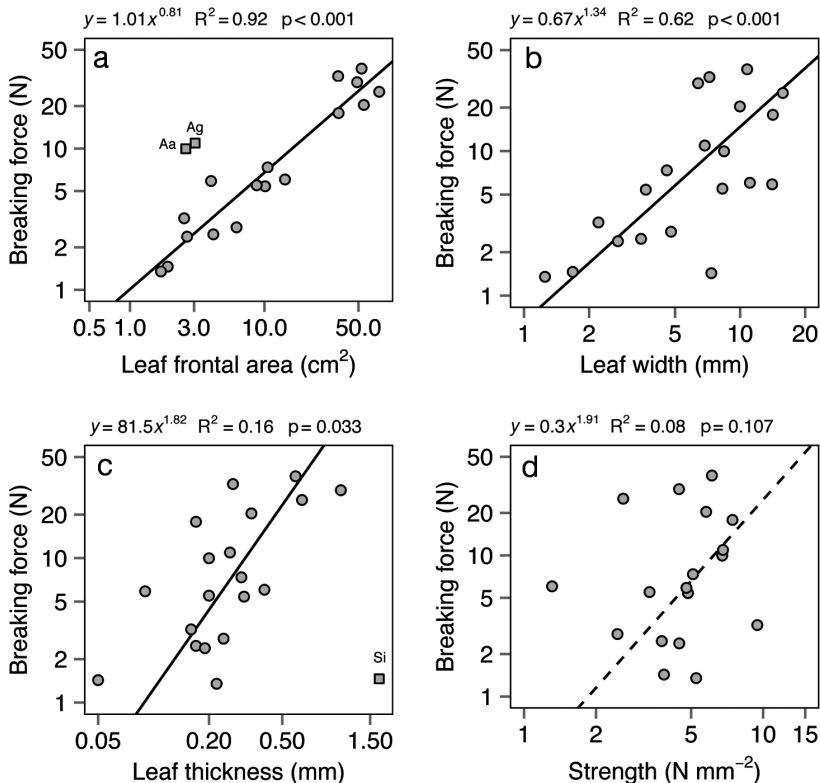


Fig. 4. Scaling relationships between leaf breaking force and leaf size (frontal area, width, and thickness) and tensile strength, with fitted lines (solid for significant relationships) from regression analyses (standardised major axis), after excluding influential species: *Amphibolis antarctica* (Aa), *A. griffithii* (Ag), and *Syringodium isoetifolium* (Si)

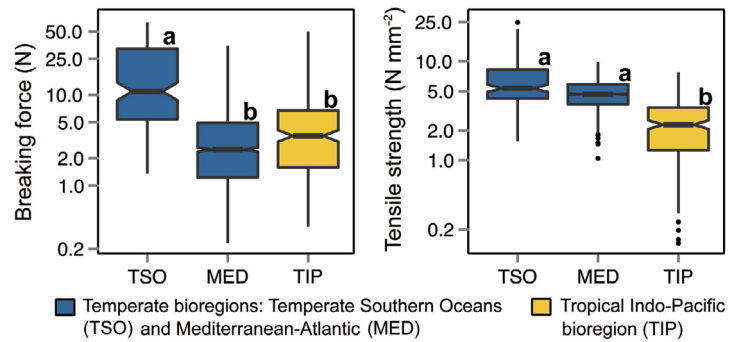


Fig. 3. Leaf mechanical properties across temperate and tropical bioregions. Boxplot bar: median; notch: 95% confidence interval around median; box: interquartile range (IQR); whiskers: max./min. values within $1.5 \times$ IQR of the hinge; dots: outliers. Notches in boxplots are used to compare values among groups and plant parts: if the notches of 2 boxes do not overlap, this is strong evidence that the medians differ. Lettering indicates homogeneous groups from multiple comparison (Tukey's test) when effect of bioregion was significant in the linear mixed-effects model ($p < 0.005$)

The analysis of variance partitioning showed that variance in leaf breaking force among species was explained by variation in leaf width (62%), thickness (20%), and tensile strength (17%) (Table 2). In contrast, intra-specific variation in breaking force was, on average, explained by variation in tensile strength (74%) followed by a similar contribution of width (15%) and thickness (11%) (Table 2).

Structural reinforcement over a range of leaf lifespans

Leaf fibre concentration (%) was fairly uniform within-species (CV 8%) and varied 2-fold among species, from 30.7 (*S. isoetifolium*) to 59.5% DW (*P. sinuosa*), whereas leaf mass per area varied 4.7-fold, ranging from 26.6 g m^{-2} (*H. ovalis*) to 124.7 g m^{-2} (*P. coriacea*) (Table S4 in Supplement 2). Species-averaged leaf lifespan varied 33-fold, ranging from 8 (*H. ovalis*) to 280 d (*P. oceanica*) (Table S2). Long-lived species presented higher mass allocated per leaf area and higher leaf fibre content, and they supported higher breaking forces than did short-lived ones (Fig. 5, Table 3). These correlations were weakened by *S. isoetifolium*,

which presented a very low leaf fibre content. The associations between structural or mechanical traits and leaf lifespan remained largely unchanged even if the phylogeny was considered. However, some correlations with leaf fibre concentration became less strong and insignificant after phylogeny was considered (Table 3).

Pairs of traits in seagrass species aligned with global datasets (Wright et al. 2004, Onoda et al. 2011)

Table 2. Within- and among-species proportion per unit of variance in leaf breaking force explained by leaf width, thickness, and tensile strength. For each species, components contributing greater than one-third to total variance of breaking force are highlighted in **bold**. Only species with at least 10 replicates were included in the within-species analysis. n: number of observations.

Species	n	Leaf tensile strength	Leaf thickness	Leaf width
Within-species				
<i>Amphibolis antarctica</i>	20	1.451	-0.729	0.278
<i>Amphibolis griffithii</i>	10	1.716	-0.654	-0.062
<i>Cymodocea nodosa</i>	170	0.273	0.406	0.321
<i>Cymodocea rotundata</i>	29	0.919	0.071	0.010
<i>Cymodocea serrulata</i>	27	0.764	0.060	0.176
<i>Enhalus acoroides</i>	45	0.727	0.121	0.152
<i>Halodule uninervis</i>	33	0.432	0.344	0.223
<i>Halophila ovalis</i>	36	0.479	0.333	0.187
<i>Posidonia australis</i>	44	0.951	-0.076	0.124
<i>Posidonia oceanica</i>	13	0.330	0.692	-0.021
<i>Posidonia sinuosa</i>	12	0.642	0.323	0.036
<i>Ruppia maritima</i>	10	0.776	0.054	0.170
<i>Syringodium isoetifolium</i>	34	1.276	-0.138	-0.138
<i>Thalassia hemprichii</i>	45	0.727	0.163	0.110
<i>Zostera capricorni</i>	10	0.229	0.213	0.558
<i>Zostera marina</i>	27	0.392	0.434	0.175
<i>Zostera noltei</i>	157	0.446	0.277	0.277
Within-species (average)	17	0.737	0.111	0.152
Among-species (overall)	744	0.174	0.195	0.621

Table 3. Correlation coefficients (r) for combination of seagrass leaf traits based on species-averaged log₁₀-transformed data, across-species (Pearson) and using phylogenetic independent contrasts (PICs)

Leaf trait combination		— Pearson —			— PICs —		
		r	p	df	r	p	df
Leaf mass per area	Fibre content	0.350	0.130	18	0.256	0.291	17
Leaf mass per area	Fibre content [‡]	0.482	0.036	17	0.319	0.198	16
Leaf mass per area	Leaf lifespan	0.672	0.002	16	0.701	0.002	15
Leaf mass per area	Breaking force	0.645	0.002	18	0.660	0.002	17
Fibre content	Breaking force	0.749	<0.001	18	0.592	0.008	17
Fibre content	Leaf lifespan	0.489	0.040	16	0.343	0.178	15
Fibre content	Leaf lifespan [‡]	0.697	0.002	15	0.621	0.010	14
Leaf lifespan	Breaking force	0.687	0.002	16	0.677	0.002	15

[‡]Excluding terete-leaved *Syringodium isoetifolium*

of terrestrial plants (Fig. 6). Overall, seagrasses tend to have shorter leaf lifespan and lower leaf mass area compared to terrestrial plants, (Fig. 6a), but their leaf strength (Fig. 6b) and fibre content (Fig. 6c) were, on the whole, in the middle part of the global leaf spectrum, especially when compared to herbaceous land plants.

DISCUSSION

Variation among species, growth-forms and bioregions

The analysis of mechanical and structural traits in one-third of seagrass species around the world revealed that, among species, breaking force, cross-section area and leaf surface area varied 50-, 85- and 123-fold. This variation was substantially larger than inter-specific variation in material properties such as strength (20-fold), extensibility (5-fold) and stiffness (23-fold). The wide variation in breaking force and leaf dimensions among species may reflect variation in the mechanical requirements of living in contrasting environments in which they occur, where they face a diverse range of hydrodynamic forces and other factors such as light regime and nutrient availability. With such a diversity of environments, species present particular combinations of growth-form, size and leaf mechanical and structural traits. On the whole, species resisting low forces (e.g. *Zostera noltei*, *Ruppia maritima*, *Syringodium isoetifolium*, *Halophila* spp., *Halodule* spp.) are small-sized, and most of them are normally found in the intertidal zone

of sheltered locations such as estuaries or subtidally in the understory, sheltered by bigger species. Their small leaf size is likely to be beneficial in retaining water by lying on the sediment surface and avoiding too much desiccation stress at low tide (Hemminga & Duarte 2000). The most resistant species (*Posidonia* spp., *Enhalus acoroides*, *Thalassodendron pachyrhizum*, *Amphibolis* spp.) typically occur subtidally in areas often exposed to strong water movement, and they are characterised by hav-

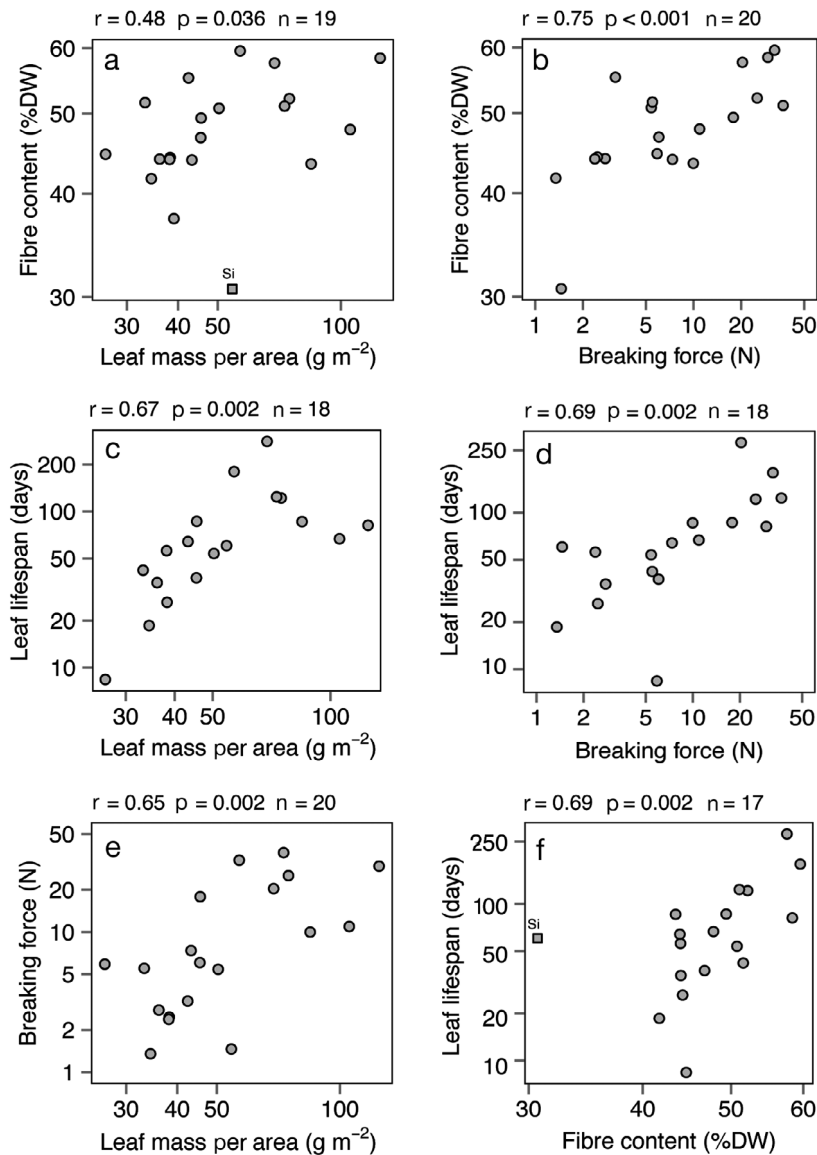


Fig. 5. Correlations among leaf mass per area, lifespan, breaking force, and fibre content. Influential species *Syringodium isoetifolium* (Si) was excluded from panels (a) and (f)

ing long strong leaves (e.g. *Posidonia* spp.) or tough stems with a cluster of leaves (normally reduced in size, e.g. *Amphibolis* spp.) at the apex.

The 3 seagrass growth-forms did not show clear patterns in leaf mechanical properties or breaking force, due to the variation in these traits within the most common growth-form, i.e. strap-like leaves. Instead, the most variable features among growth-forms were found in the non-foliar tissues. The stems of *A. antarctica*, *A. griffithii*, *T. pachyrhizum*, and *Z. nigricaulis* were extremely strong and extensible, attributes making them good energy absorbers, a beneficial feature in high-energy environments

(Koehl 1982, 1984). Similarly, petioles of the *Halophila* spp. were very stretchy (50–58% extension before the breakage), which also made them able to absorb energy before breaking.

In contrast to the global patterns in leaf mechanical properties of terrestrial species, where no clear differences occur between tropical and temperate leaves (Onoda et al. 2011), we found that leaves of temperate seagrasses were generally more resistant than leaves of tropical ones among the selected species and sites, except for the tropical species *Enhalus acoroides*, which ranked amongst the strongest species. This pattern could be a requisite to face the high hydrodynamic forces that, in general, temperate species experience in their habitat, especially those from the Temperate Southern Oceans, where extensive meadows are often growing in habitats exposed to strong wave and current force (Carruthers et al. 2007). Tropical seagrass beds, in contrast, normally occur in lagoons between barrier reefs and mainland, where they are protected from waves (Carruthers et al. 2007). In addition to hydrodynamics, other factors could be behind this pattern of leaf breaking force of seagrasses among bio-regions, such as light and nutrient regimes, which might influence leaf dimensions or the availability of resources.

The relatively soft leaves that we observed in most tropical species may explain why they are an important food source for sirenians and sea turtles, especially in historical times when the high abundance of these herbivores severely cropped seagrass meadows (Valentine & Duffy 2006). Some of the temperate seagrasses from the Mediterranean–Atlantic bioregion, especially *Z. marina*, are also heavily grazed by the once abundant herbivorous waterfowl (Valentine & Duffy 2006). Conversely, temperate Indian Ocean species are commonly subject to very low grazing rates (reviewed in de los Santos et al. 2012). This is possibly explained by the tough leaves that seagrasses generally have in the

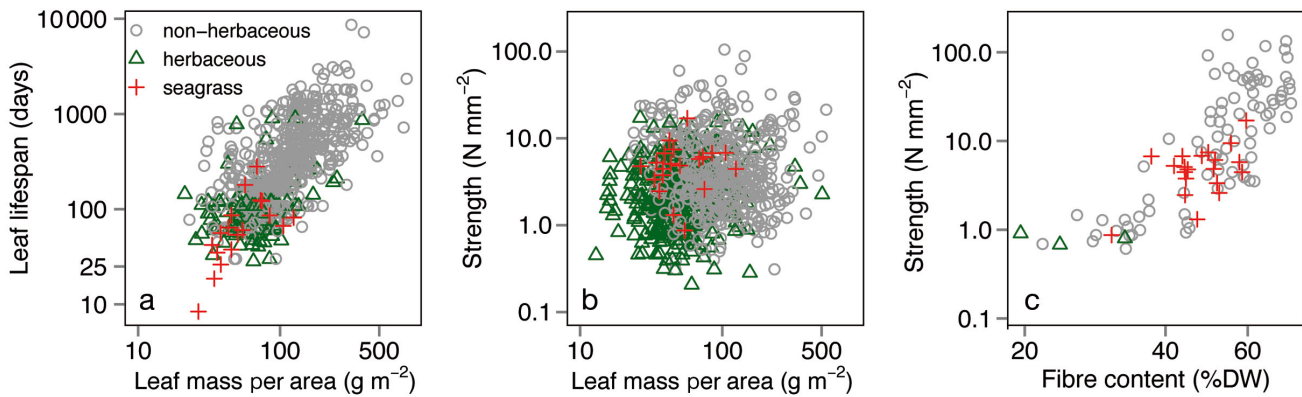


Fig. 6. Correlation of leaf lifespan, leaf mass per area, leaf strength and fibre content in seagrasses and terrestrial plants (herbaceous and non-herbaceous) using (a) the global data set from Wright et al. (2004) and (b,c) data compiled in Onoda et al. (2011)

Temperate Indian Ocean bioregion (Fig. 3). The higher leaf breaking force of temperate species, compared to the tropical ones, correlates with longevity, as reported here and by Hemminga et al. (1999).

Contribution of morphology to breaking force and scaling relationships

Variation of breaking force among species was mostly explained by leaf width, rather than by thickness or tensile strength. As explained in the 'Introduction', the different ways to attain a high breaking force, either by thickness, width or strength, are accompanied by different costs for the plants.

The low contribution of leaf thickness to breaking force may be because leaf thickening notably increases the leaf flexural stiffness, which is proportional to thickness to the third power (Niklas 1992), and being stiff subtracts capacity to streamline with the flow, thus increasing the experienced drag force (Bouma et al. 2005). Leaf thickening may also be a disadvantage for high photosynthetic performance (Agustí et al. 1994) because it reduces the surface area per unit mass to intercept light, which is critical in the frequently light-limited conditions in the marine environment.

Leaf widening contributes to breaking force by increasing the cross-section area but it does not increase flexural stiffness as much as thickness because the latter is proportional to width to the first power. However, leaf widening increases the frontal area and thereby the potential drag force. In principle, the drag force is proportional to the surface area if other variables are constant and if bending does not occur. In case bending does occur, increases in hydrodynamics from flow or waves have relatively

little additive effect on drag forces (Bouma et al. 2005, 2010). Moreover, as leaf breaking force scales to frontal area (length \times width) with a scaling slope less than 1, bigger leaves also do not experience the risk of breaking from drag forces in proportion to their size. In addition, wide (and long) leaves increase the surface area for light interception and have a competitive advantage in light competition against other individuals and species. The strong positive correlation between breaking force and leaf surface area may indicate that a high breaking force is essential for long leaves to tolerate the drag forces.

The variation of breaking force among species was poorly explained by variation in tensile strength, because cross-species variation in leaf tensile strength was rather limited compared to the variations in morphological traits. The limited variation in tensile strength may be due to a strong selection pressure over evolutionary time on leaf properties in aquatic environments. In turn, this suggests that most of the seagrass diversification in terms of tolerance to physical forces is related to morphological features rather than material properties. In summary, the most common leaf form selected over the transition of angiosperms back to the sea is the long and thin leaf form, with some variation in their width, likely due to benefits for leaf reconfiguration under strong hydrodynamic force in combination with adequate light interception for growth.

Structural reinforcement along a range of leaf lifespans

The phylogenetic independent contrast analyses showed that the associations among mechanical (breaking force) and structural (leaf mass per area)

traits and leaf longevity have repeatedly evolved in different clades of seagrasses, rather than being due to a phylogenetic bias in the studied species. These findings are in accordance with the leaf economic spectrum (Reich et al. 1991, Wright et al. 2004) and mirror global patterns described in land plant species (Onoda et al. 2011). In particular, seagrass species are located at the low end of the spectrum; that is, seagrasses can be globally considered as short-lived plant species (Hemminga et al. 1999) with a low leaf mass per unit area. This finding widens the extent of leaf economics spectrum to the seagrasses showing their unique position along the spectrum in comparison to terrestrial plants. The lower leaf lifespan of seagrasses is likely explained by rapid colonisation of seagrass leaves by epiphytes, which leads to a decrease in functionality in a short period of time. Epiphytes may also lead to increased drag forces by enhancing the roughness of the leaf area and reducing the capacity to reconfigure. In addition, leaves in many seagrass environments are subjected to continuous physical disturbance, which may accelerate the leaf abscission, especially in old leaves, which become significantly weaker as they age (de los Santos et al. 2016). Furthermore, aquatic plants are free from the costs of enduring long desiccation and strong gravity, so that seagrass leaves would require a lower dry mass investment, i.e. smaller leaf mass per area.

Despite their low leaf mass area, seagrass leaves were moderately strong and presented a high proportion of leaf fibres in comparison to most terrestrial plants (Fig. 6). In fact, many seagrass species examined here (especially the genera *Posidonia*, *Enhalus* and *Cymodocea*) presented high proportions of leaf fibres (30–60%, average 47.7%). These values are similar to the mean value for the terrestrial counterparts (47.3%) but substantially higher when exclusively compared to terrestrial herbaceous plants (25.9%; Onoda et al. 2011). The interspecific variation of the fibre concentration in seagrass may depend partly on the lineages as shown by the lack of significant correlations using PICs. The high proportion of fibres in the seagrass leaves and their high strength might suggest that leaf reinforcement, by fortifying support tissues, is a requisite to the marine existence.

Seagrass biomechanics and ecology

Knowledge of plant biomechanics is essential to understand how they respond and interact with the

physical forces (both abiotic and biotic) encountered during their lifespan (Read & Stokes 2006). Seagrasses are consumed by many animal groups (e.g. fishes, sea urchins, mammals, turtles, waterfowl, crustaceans, gastropods; Valentine & Duffy 2006) with variable feeding techniques such as leaf biting or mining. Thus, seagrass leaves are potentially exposed to the forces exerted by the feeding apparatus of those herbivores, which have to overcome the breaking strength of the seagrass leaves for a successful intake. The database on seagrass breaking force (compiled during the present study) is therefore ecologically relevant to study plant–herbivore interactions. For instance, the bite force of the green turtle *Chelonia mydas* ranges from 123 to 303 N (Marshall et al. 2014), which is much higher than the maximum seagrass breaking force reported in our study (63.7 N in a sample of *Posidonia australis*). Thus, green turtles could potentially graze on all of the assessed species, although other factors should be also considered, such as the leaf nutritional value of the different species, which is tightly correlated to mechanical strength (de los Santos et al. 2012).

Regarding abiotic forces, seagrasses are continuously exposed to a complex combination of drag and acceleration forces as a result of the action of waves and currents. The breakage of seagrass leaves is ultimately controlled by the balance between those hydrodynamic forces and the leaf breaking force. Indeed, the ratio of these 2 forces (the so-called 'safety factor'; Niklas 1992) is a measure of leaf mechanical reliability. Our database (present study) on leaf breaking force will therefore be useful to predict whether a seagrass leaf or structure (e.g. stem) will break or not under a given hydrodynamic force. Unfortunately, forces exerted on seagrass leaves in their habitat are difficult to measure and such studies are virtually absent from the literature (Koch et al. 2006). Further research should include *in situ* measurements of forces, or force predictions from hydro-mechanical models (as in Friedland & Denny 1995); quantifying seagrass mechanical limits will thus allow evaluation of the safety margins for mechanical stability in their hydrodynamically active environment.

CONCLUSIONS

Our analysis of mechanical and morphological traits in one-third of the world's seagrass species, and the comparison with terrestrial plants, provide insights into seagrass requirements to face the

physical forces in the marine environment. Temperate species were, on average, stronger than tropical, possibly because seagrasses are generally subjected to stronger hydrodynamic forces in temperate environments than in tropical ones. Secondly, we found that the tensile strength of seagrass leaves was very similar among species and that the large variation in leaf breaking force is largely explained by differences in morphology, especially leaf width. Based on that, we conclude that, among seagrass species, widening the leaves is likely the most effective way to tolerate higher forces, since other options, such as leaf thickening, may compromise the leaf flexibility or the efficiency for light interception. Finally, the similarities of trait intercorrelation with land plants indicate that common ecological processes acted on land and aquatic environments during evolutionary time, although the strong influence of hydrodynamics in seagrasses could be responsible for their moderately strong fibre-reinforced leaves of short life-span.

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