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New aspect in seagrass acclimation: leaf mechanical properties vary spatially and seasonally in the temperate species *Cymodocea nodosa* Ucria (Ascherson)

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Abstract Seagrasses may acclimate to environmental heterogeneity through phenotypic plasticity. In contrast to leaf morphology, which has been a central point in seagrass acclimation studies, plasticity in leaf biomechanics and fibre content is poorly understood, despite being crucial in plant ecological performance, especially regarding physical forces. We hypothesised that mechanical traits (e.g. breaking force, strength, toughness, and stiffness) and fibre content of seagrass leaves vary as morphology does under differential environments. *Cymodocea nodosa* was seasonally monitored at three locations around Cádiz Bay (southern Spain) with hydrodynamic regime as the most noticeable difference between them. Leaves showed plasticity in both morphology and mechanical traits, with wave-exposed individuals presenting short but extensible and tough leaves. Leaf fibre content was invariant along the year and with little spatial variability. Cross-sectional area rather than material properties or fibre content differentiates leaf mechanical resistance. Seagrass capacity to thrive under a range of mechanical forces may be dictated by their plasticity in morpho-biomechanical traits, a key element for the hydrodynamical performance and, hence, for species colonisation and distribution.

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Introduction

Seagrasses are clonal plants living in shallow coastal waters, exposed to high environmental variability. In heterogeneous environments, phenotypic plasticity (i.e. the potential ability of an organism to change its phenotype in response to environmental forcing) may be beneficial (Sultan 1987; Pigliucci 2001), although it may also be costly and limited (DeWitt et al. 1998). In seagrasses, the well-documented and noticeable phenotypic plasticity allows these organisms to acclimate to environmental heterogeneity and cope with environmental stresses (Hemminga and Duarte 2000). The magnitude of the intraspecific variability for a plant trait, normally quantified as the coefficient of variation within species (Marbà and Duarte 1998; Brun et al. 2006), reflects seagrass capacity to colonise and survive to environmental changes (Marbà and Duarte 1998) and may explain species distribution (Mascaró et al. 2009).

The environmental variability manifests at spatial and/or temporal scales, being the latter especially noteworthy in temperate regions, where seagrasses are subjected to seasonal changes. This variability mainly includes changes in light and nutrient availability, but hydrodynamic regime, emersion, burial/erosion episodes, and other factors may have a strong influence on the plants. Temperate seagrasses normally respond to these multiple factors through changes in morphological, dynamical, and biochemical traits; resource allocation; and physiological rates (e.g. Dennison and Alberte 1986; Pérez et al. 1994; van Lent and Verschuure 1994; Vermaat and Verhagen 1996; Sfriso and Ghetti 1998; Marbà and Duarte 1998; Laugier et al. 1999; Peralta et al. 2000, 2005, 2006; Pergent-Martini et al. 2005; Lee et al. 2005; Cunha and Duarte 2007; Olivé et al. 2007, 2012; Cabaço et al. 2009; Mascaró et al. 2009). In addition

to these plant traits, leaf mechanical traits should be also investigated, as they are fundamental for plant hydrodynamical performance and fitness (Read and Stokes 2006) as well as for seagrass ecosystem functions (Peralta et al. 2008; Bouma et al. 2009; de los Santos et al. 2012).

Plant biomechanical features have been normally related to the mechanical environment and the resource supply they encounter (Kopp 1999; Read and Stokes 2006; Onoda et al. 2008; La Nafie et al. 2012; Lamberti-Raverot and Puijalon 2012). Being exposed to water motion, aquatic plants experience complex mechanical loads, especially drag forces, which deform them and may eventually result in breakage or uprooting (Koehl 1982; Vogel 1996; Schutten et al. 2004; Nikora 2010). The plant's capacity to resist these forces will determine the species persistence in their habitat. This capacity depends on leaf biomechanical traits such as strength, stiffness, toughness, and extensibility, which rely on the leaf structural reinforcement, material properties, and morphology. The potential to resist mechanical forces may be accomplished through plant adaptation (in evolutionary terms) or acclimation (by phenotypic plasticity), either by minimising the forces encountered ('avoidance strategy') or by maximising their resistance to breakage ('tolerance strategy') (Vogel 1984; Denny and Cowen 1997; Ennos 1999; Sand-Jensen 2003; Bouma et al. 2005; Puijalon et al. 2008; Bornette and Puijalon 2011). The investigation of those capacities is consequently a key element for predicting population structure and distribution under mechanically stressful conditions.

Seagrasses acclimate to physical forces (i.e. water motion) by adjustments in morphological and dynamical traits (Schanz and Asmus 2003; Peralta et al. 2005, 2006; de los Santos et al. 2010; La Nafie et al. 2012). For instance, *Zostera noltii* acclimates to water flow through changes in morphology and biomass allocation, being the leaves shorter and narrower at high flow velocities (Peralta et al. 2005, 2006; de los Santos et al. 2010). In addition to morphology, biomechanical properties of the foliar tissues, as aforementioned, are crucial in coping with the physical forces (Read and Stokes 2006) and plants may present a heterogeneous profile of their mechanical traits under different physical environments (Niklas 1992; Anten et al. 2010). Acclimation in biomechanic traits has been investigated in macroalgae (mainly kelps; e.g. Duggins et al. 2003) and water moss (Biehle et al. 1998), only the former manifesting plasticity in the mechanical properties (kelp fronds resisted high forces under high flow energy regimes). However, changes in seagrass leaf biomechanical traits driven by environmental heterogeneity, especially hydrodynamics, are not well documented, with only a single study on the seasonality of leaf strength in *Zostera marina* (Kopp 1999) and an experimental study on *Z. noltii* (La Nafie et al. 2012). Variation in leaf mechanical

properties may reflect underlying variations in cellular structures, possibly via cell orientation, proportion of strengthening cell wall materials or anatomy (Biehle et al. 1998; Onoda et al. 2004; Lamberti-Raverot and Puijalon 2012). Therefore, associated costs of construction (investment in supporting tissues) are expected if seagrass leaves acclimate to environmental heterogeneity from a biomechanical point of view. In other words, changing mechanical properties of leaves may increase the ecological breadth of the species but, as carbon-based resources may be required to strengthen and toughen the supporting tissues, a trade-off may exist in resource investment into growth.

We hypothesise that environmental heterogeneity, especially hydrodynamics, not only affects plant morphology but also mechanical properties and fibre content of seagrass leaves. Thus, we expect that under high hydrodynamic conditions, that is, flow and/or waves presence, seagrass plants will acclimate by shortening leaves and also by producing more resistant leaves, that is, leaves supporting higher forces before breaking. Cádiz Bay (southern Spain) is an excellent outdoor laboratory to undertake this study, since *C. nodosa*, a temperate seagrass species, inhabits several locations with different degrees of exposure to currents and waves, showing contrasting morphologies among locations and a seasonal pattern in morphological, dynamical, and population traits (Olivé et al. 2012; Pérez-Lloréns et al. unpublished data). We explicitly hypothesised that (1) mechanical properties of seagrass leaves change to accommodate spatial and seasonal environmental heterogeneity, in a similar way that morphology does, and (2) this variation implies changes in the leaf fibre content.

Materials and methods

Sampling sites and biological material

The temperate seagrass species *C. nodosa* Ucria (Ascherson) was selected for being a species that forms perennial populations in three locations around Cádiz Bay (southern Spain; Fig. 1): Santibáñez (ST), El Bajo de la Cabezuela (BC), and El Chato (CH). Cádiz area is characterised by the typical seasonal trend for temperate climates, with maximal values of solar radiation and temperature in summer and minimal in winter (Vergara et al. 2012), and winter storm period spanning between November and March. The tidal regime in the Cádiz Bay and surroundings is mesotidal and semidiurnal, with a mean tidal range of 2.3 m and mean spring tidal range of 3.7 m (del Río et al. 2012), with the amplitude of the tide increasing from the mouth of the outer bay towards its interior (Álvarez et al. 1997). Both

Fig. 1 Map of the study area and the sites where *C. nodosa* was sampled (36°28′N–6°15′W). Locations (from high to low exposure): CH, El Chato (high exposure to waves, intertidal); BC, Bajo de la Cabezuela (medium exposure to waves and currents, subtidal); ST, Santibáñez (low exposure to waves and currents) with two stations: intertidal (ST-int) and subtidal (ST-sub)
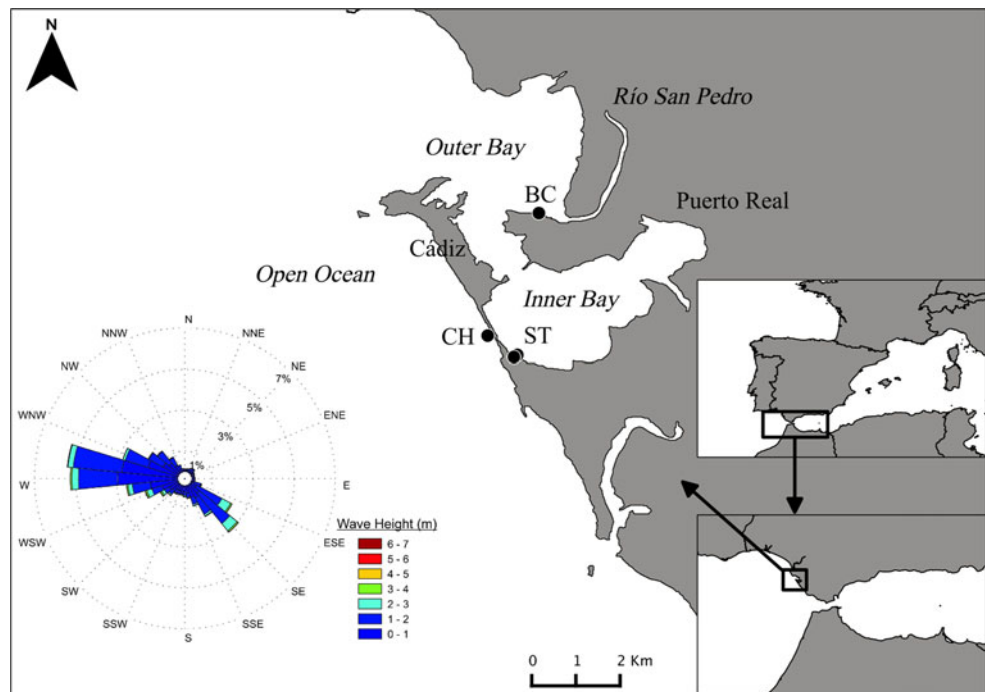
sea and swell waves generally approach the coast of Cádiz from the W and SW, giving rise to a prevailing longshore current towards the E and SE (del Río et al. 2012; Fig. 1).

Santibáñez saltmarsh (ST, 36°28′09.08″N—06°15′04.64″W) is located in the southern point of the inner sector of Cádiz Bay, a shallow tidal lagoon (3 m mean depth with respect to the mean low water) with wide tidal flats and a complex system of tidal creeks (Fig. 1). Sediment within the inner bay is composed mainly of fine sand and mud, with high organic carbon content (Carrasco et al. 2003; Rueda and Salas 2003). The typical waves in Cádiz Bay are short-period wind waves (mean significant wave height of 0.6 m); however, the inner bay, and specially the site of collection, is protected from the action of large waves, so that the wave height is below the averaged value. The mean velocity module during a tidal cycle ranges from 0.015 to 0.08 m s^{-1} (Lara et al. 2012), which is expected to increase when strong eastern winds blow and during winter time (Kagan et al. 2003). *Cymodocea nodosa* inhabits the shallow, low-current velocity, south-western area at both the intertidal fringe (ST-int) and as continuous monospecific meadows at the subtidal zone (ST-sub), at 0.4 and −0.5 m above and below the chart datum (lowest astronomical tide), respectively (Olivé et al. 2012). *Cymodocea nodosa* populations in Santibáñez exhibit an unimodal seasonal pattern of growth, with maximum growth rates and shoot sizes in summer (Pérez-Lloréns et al. unpublished data). The shoot density and biomass in this area vary with tidal position: intertidal meadows have an annual average shoot density of 1194 ± 513 shoots m^{-2}

and an annual average leaf biomass of 86 ± 77 g dry weight m^{-2}, whereas those from the subtidal present an annual average shoot density of 447 ± 174 shoots m^{-2} and leaf biomass of 120 ± 78 g dry weight m^{-2} (mean ± SD, $n = 48$; averaged for 2009 and 2010; Pérez-Lloréns et al. unpublished data).

El Bajo de la Cabezuela (36°31′42.52″N—06°14′32.16″W) is a shallow inlet located at the 'Río San Pedro' salt marsh and comprises a tidal sound with its wider mouth opened to outer Cádiz Bay and the other artificially closed upstream (Fig. 1). This sandflat is characterised by semidiurnal mesotides with the tidal current flowing from the bay along the inlet. Plants were collected at the mouth of the inlet, a location subjected to high currents powered by tide, and short-period wind-generated waves (which increased during winter), and recurrent episodes of sediment erosion and accretion (Kagan et al. 2003, 2005; Brun et al. 2005). Mean velocity module measured 10 cm above the sea floor during a tidal cycle ranged from 0.05 to 0.25 m s^{-1} (Lara 2012), while wave heights varied from 0 m to 0.15–0.20 m on windy days (Kagan et al. 2005). This sandflat is colonised by continuous beds of *C. nodosa* in the shallow subtidal zones, with an average shoot density of 1191 ± 107 shoots m^{-2} and an average aboveground biomass of 142 ± 33 g dry weight m^{-2} (González-Ortiz 2009).

El Chato (36°28′38.16″N—06°15′49.21″W) is a shore-line-parallel rocky outcrop located at the Cortadura beach, facing the open ocean with a NNW–SSE orientation (Fig. 1). The rocky platform has a maximum width of 100–200 m (1 to 1.5 above chart datum) and presents several intertidal pools with sandy accumulation on the

bottom (medium sand; averaged depth of 0.20 ± 0.10 m; Velasco 2006). The hydrodynamic conditions of this location highly differ from the others as it is the only location exposed to open ocean, thus exposed to strong currents (data not available) and large waves reaching and breaking on the rocks (mean annual significant wave height 1.13 m and maximum of 5.40 m; Puertos del Estado 2009). A high diverse community of macroalgae thrives on this highly exposed habitat, being *C. nodosa* the only existing seagrass species, which present a strong anchorage system to attach to the bottom. *Cymodocea nodosa* forms small scattered patches of very low shoot density (data not available, but almost isolated shoots) on the sand accumulations in the bottoms of the rocky pools.

Since all the locations where the plants were collected are within an area of 20 Km^2, strong differences in climate and hydrology are not expected. The most noticeable abiotic factor differentiating the three locations is the hydrodynamic regime, as they have contrasting wave exposure (since they have different spatial orientation, thus different fetch) which is strongly correlated with currents. Therefore, seagrass meadows from each location are exposed to different mechanical loads, with a gradient of exposure to waves and currents ranging from Santibáñez (low exposure), Bajo de la Cabezuela (medium exposure), to El Chato (high exposure). In each location, *C. nodosa* shoots were collected between summer 2009 and spring 2010 (mid July 2009, early November 2009, early March 2010, end May 2010). Entire specimens were manually and randomly collected by wading on the shore and sampling from different patches with a distance of >10 m from each other to avoid pseudoreplication. Plant material was handled carefully after collection to minimise changes in their turgidity and physical integrity, and it was immediately and carefully transported to the lab in wet, cool, and dark conditions. Plants were kept in tanks with aerated seawater at climate room temperature (20 °C) until being tested (within 48 h from collection).

Leaf morphology

Prior to any measurements, specimens were carefully selected from the collected material, discarding flawed or nicked specimens, which would underestimate their mechanical properties, and selecting only those with healthy appearance. Also, the bulk of epiphytes was removed carefully. Specimens were submerged into seawater at room temperature (20 °C) until testing, when they were cut into fragments. The first unbroken, fully developed leaf of the shoot (normally the second outermost leaf) was selected, and a leaf blade fragment from 20 to 50 mm in length (L_0) was excised from the junction between the blade and the sheath in the direction of the leaf tip.

Morphological traits were measured before mechanical tests, including leaf width (Wd, mm) and thickness (Th, mm) for the calculation of the cross-sectional area (CA, mm^2; CA = Th $\times$ Wd), and total leaf length (LL, m). The entire volume (V, mm^3) of each fragment was estimated according to the geometrical approximation of a rectangular cross-sectional area ($V = $ CA $\times L_0$).

Leaf biomechanics

Tensile properties of the seagrass leaf blades were measured with a tensometer (Instron® universal testing machine, model 3340) and the BlueHill® software (v. 2.18), using load cells of 10 or 100 N, and pneumatic action grips of 5 or 250 N (model 2712). Tests were conducted in leaf fragments in parallel to its main axis, which were clamped individually into the grips of the tensometer with the mountings 20–40 mm apart (depending on the leaf size), with the exact distance measured to the closest 1 mm. Once clamped in the grips, the tissue fragment 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 s until breakage, when a maximum force (absolute force-to-tear, F_{TA}, N) and displacement (δ_T, mm) were recorded. From the force–displacement curve and the morphological traits of the specimens, the following mechanical properties were obtained (Table 1): (a) absolute force-to-tear (F_{TA}, N), the maximum force that the specimen can bear before breaking; (b) specific force-to-tear (F_{TS}, N mm^{-2}), the maximum force per unit of cross-sectional area needed to break the specimen. This is a property known as 'tensile strength' or 'breaking stress' in engineering (Gere and Goodno 2012); (c) elongation-to-tear (L_T, %), which correspond to the increase in length ($L_0 + \delta_T$) relative to the original length of the leaf portion (L_0) that occurs before it breaks. This property is also called 'ultimate elongation' or 'breaking strain' in engineering (Gere and Goodno 2012); (d) Young's modulus of elasticity in tension (E_T, N mm^{-2}), that is, the resistance to deformation or material stiffness. The modulus was calculated by taking the initial, computer fitted gradient from the force against displacement graph (F/δ) as well as considering the initial length (L_0) and the cross-sectional area of the specimen (CA). The higher the E_T, the stiffer the material; (e) absolute work-to-tear (W_{TA}, mJ) is the work required to break the tissue. It was calculated as the area under the force-extension curve; (f) specific work-to-tear (W_{TS}, mJ mm^{-3}) is the work-to-tear (W_{TA}) normalised by the tissue volume, and it is also called 'tensile toughness'. Tests were made on 5–15 replicates per each location and season, excluding the specimens that slipped during the test or broke at the grips.

Table 1 Mechanical traits, their abbreviations (abbr.), units, interpretation, and computation

Abbreviations	Trait	Units	Meaning and interpretation	Equation
Whole-tissue mechanical traits				
F_{TA}	Absolute force-to-tear	N	Total amount of force needed to break a leaf	
W_{TA}	Absolute work-to-tear	mJ	Total amount of work needed to break a leaf	
Material mechanical traits				
F_{TS}	Specific force-to-tear	N mm^{-2}	Also termed 'tensile strength' or 'breaking strength'. Force required per unit of cross-sectional area to break a leaf. The higher the tensile strength, the higher the material resistance	$F_{TS} = \frac{F_{TA}}{CA}$
W_{TS}	Specific work-to-tear	mJ mm^{-3}	Also termed 'tensile toughness'. Work required per unit of leaf volume to break the leaf. The higher the tensile toughness, the tougher the material	$W_{TS} = \frac{W_{TA}}{V}$
E_T	Modulus of elasticity in tension	N mm^{-2}	Also termed 'material stiffness in tension'. It is the resistance to be deformed in tension. The higher the modulus, the stiffer the material	$E_T = \frac{L_0}{CA} \cdot \frac{F}{\delta}$
L_T	Elongation-to-tear	%	Also termed 'ultimate elongation' or 'breaking strain' or 'extensibility'. It is the capacity of linear deformation. The higher the elongation-to-tear, the higher the capacity of elongation before breakage (stretchy materials)	$L_T = \frac{L_0 + \delta_T}{L_0} \times 100$

F_{TA} and W_{TA} are directly obtained from the tensometer; CA, leaf cross-sectional area; V, leaf volume

Fibre content analysis

The tested leaf fragments were freeze dried and pulverised in a mixer mill to determine the neutral detergent fibre content (NDF) (modification from Van Soest et al. 1991). Three to six replicates were made for each combination of location and season. Samples of 20–30 mg of dry biomass were heated to boiling (100 °C) in 2 mL of neutral detergent during 1 h, followed by centrifuging (4,000 rpm, 5 min). The pellet was kept in the tube, washed, and centrifuged (4,000 rpm, 5 min) with distilled water (2×), ethanol (2×), and acetone (1×). The final pellet, free of non-cell wall components and chlorophyll, was dried overnight in the oven at 60 °C and weighed again. The amount of fibre in each sample was obtained by difference in mass and expressed as fibre percentage in dry biomass (% or g of fibre per 100 g of dry biomass). Neutral detergent fibre content (NDF) is referred as 'fibre content' hereafter.

Data analysis and statistics

Mean values are represented with 95 % confidence intervals. The degree of intraspecific plasticity of leaf traits was quantified by the coefficient of variation (CV). The relative contribution of leaf thickness and width to the cross-sectional area was determined from its covariance taken as a proportion of the variance of CA (Onoda et al. 2011). Significant differences between locations and between seasons within each location (interaction) were investigated using a two-way ANOVA followed by post hoc multiple comparisons (Tukey HSD), after testing homogeneity of variances (Levene's test) and normality (Shapiro–Wilks test). When necessary, variables were log_{10} transformed to meet ANOVA assumptions. When ANOVA assumptions were not satisfied after data transformation, Kruskal–Wallis nonparametric test was used followed by multiple comparisons (Behrens–Fisher test). Significance level was considered at $p < 0.05$. Statistical analyses were computed with R 2.11.1 (R Development Core Team 2010).

Results

Spatial and seasonal variation in morphological traits

Morphological variation of leaves was strongly coupled with the tidal position of *C. nodosa* populations. Subtidal individuals from El Bajo de la Cabezuela (0.46 ± 0.16 m) and Santibáñez (0.55 ± 0.12 m) presented longer leaves than intertidal individuals from El Chato (0.10 ± 0.03 m) and Santibáñez (0.27 ± 0.10 m) (Table 2; Fig. 2a). Leaf length decreased during wintertime in both subtidal populations of El Bajo de la Cabezuela and Santibáñez ($H_3 = 13.6$ and $H_3 = 9.8$, $p < 0.05$, respectively), whereas it was invariant along the year at the intertidal ones ($H_3 = 6.91$ and $H_3 = 7.7$, $p > 0.05$, for El Chato and Santibáñez, respectively). Cross-sectional area varied among locations, and leaves from subtidal populations revealed higher cross-sectional area than the intertidal ones (Table 2; Fig. 2b). Leaf cross-sectional area reflected seasonality in populations from El Chato ($H_3 = 12.8$, $p < 0.01$), Bajo de la Cabezuela ($H_3 = 18.8$, $p < 0.001$),

Table 2 Statistical results from one-way Kruskal–Wallis (H) or two-way ANOVA (F) tests

Variable	Season	Location	Season * location	CV
Leaf length (LL)	$H_3 = 1.2$ ns	$H_3 = 78.8$***	na	62
Leaf thickness (Th)	$H_3 = 10.6$*	$H_3 = 58.5$***	na	35
Leaf width (Wd)	$H_3 = 12.9$**	$H_3 = 80.1$***	na	31
Cross-sectional area (CA)	$H_3 = 1.7$ ns	$H_3 = 87.7$***	na	55
Absolute force-to-tear (F_{TA})	$F_{3,86} = 7.3$***	$F_{3,86} = 108.6$***	$F_{9,86} = 5.3$***	50
Absolute work-to-tear (W_{TA})	$F_{3,86} = 9.0$***	$F_{3,86} = 119.9$***	$F_{9,86} = 4.2$***	80
Specific force-to-tear (F_{TS})	$F_{3,86} = 1.8$ ns	$F_{3,86} = 11.2$***	$F_{9,86} = 4.5$***	29
Specific work-to-tear (W_{TS})	$F_{3,86} = 7.9$***	$F_{3,86} = 27.1$***	$F_{9,86} = 3.9$***	118
Material stiffness tensile (E_T)	$F_{3,86} = 20.2$***	$F_{3,86} = 10.8$***	$F_{9,86} = 3.2$**	25
Elongation-to-tear (L_T)	$F_{3,86} = 29.3$***	$F_{3,86} = 37.9$***	$F_{9,86} = 4.2$***	34
Fibre content (NDF)	$F_{3,33} = 2.4$ ns	$F_{3,33} = 4.1$*	$F_{9,33} = 3.1$**	10

ns Not significant, *na* not available, *CV* coefficient of variation (%)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Fig. 2 Seasonal variation of morphological leaf traits of *Cymodocea nodosa*. Mean values are represented with confident intervals (95 %). LL, leaf length; CA, cross-sectional area. Locations (from high to low exposure): CH, El Chato (high exposure to waves, intertidal); BC, Bajo de la Cabezuela (medium exposure to waves and currents, subtidal); ST, Santibáñez (low exposure to waves and currents) with two stations: intertidal (ST-int) and subtidal (ST-sub)
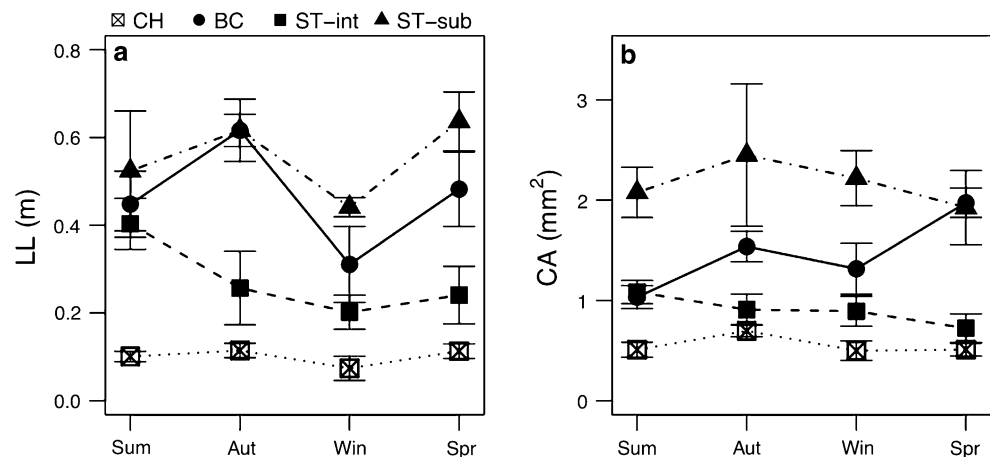
and intertidal Santibáñez ($H_3 = 8.6$, $p < 0.05$) but not for subtidal Santibáñez ($H_3 = 2.8$, $p > 0.05$). Leaf thickness had a slightly higher contribution (53 %) to the total variance in cross-sectional area than leaf width (47 %), when all the data set were considered (including seasonality and spatial variance). When the same analysis was done by location, the contribution of leaf thickness was higher in the leaves from subtidal Santibáñez (67 % thickness contribution and 33 % width contribution) and lower for leaves from Bajo de la Cabezuela (38 % thickness contribution and 62 % width contribution).
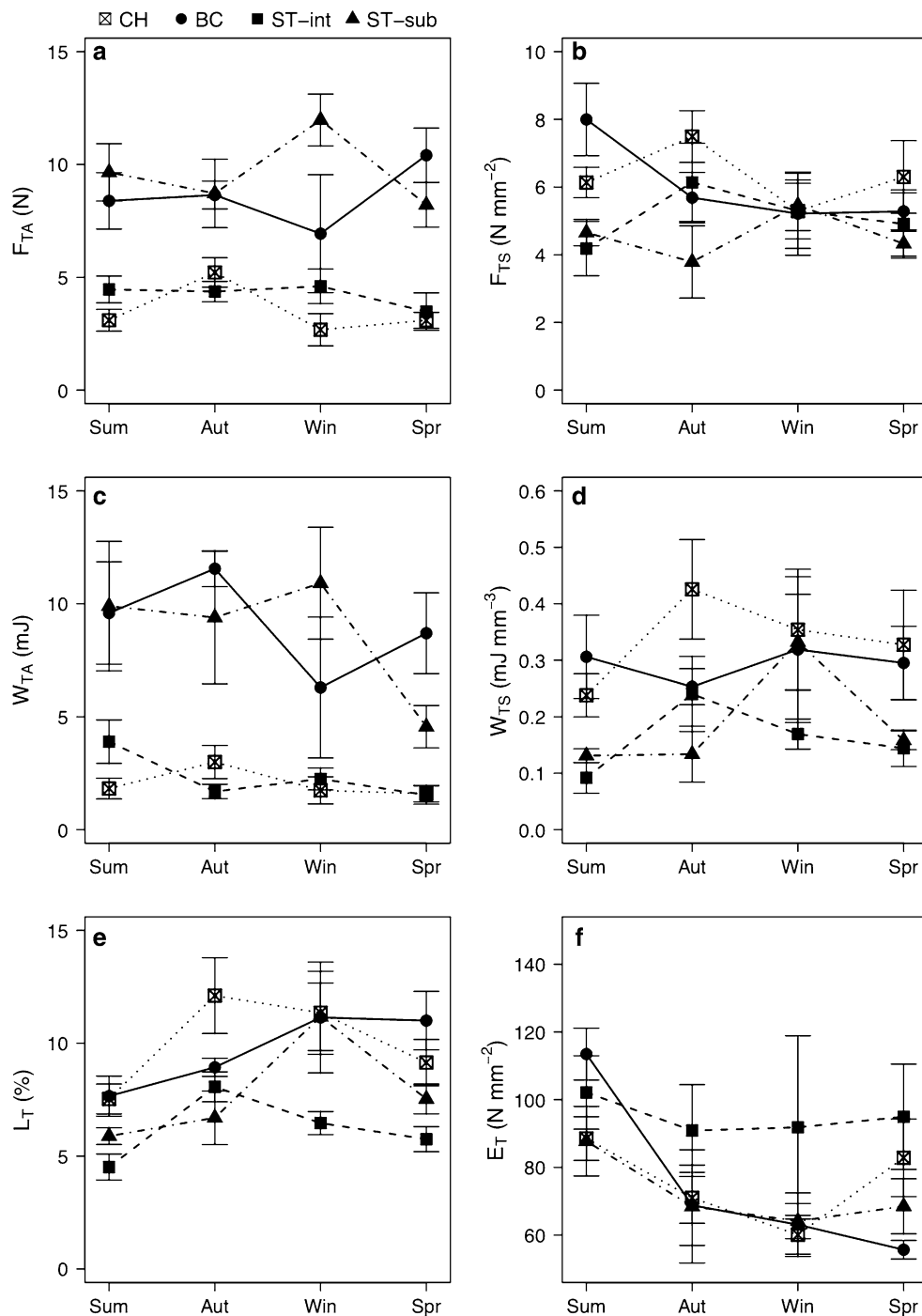
Spatial and seasonal variation in biomechanical traits

In terms of absolute force (F_{TA}) or work (W_{TA}), leaves from subtidal locations were more resistant than those from the intertidal, as these are size-dependent variables (Fig. 3a, c). When considering material properties (size-independent), plants from El Chato (high exposure to waves) and El Bajo de la Cabezuela (middle exposure to currents and waves) presented more extensible leaves (elongation-to-tear, L_T; Fig. 4c), with higher tensile strength (specific force-to-tear F_{TS}, Fig. 4a) and higher

tensile toughness (specific work-to-tear, W_{TS}, Fig. 4b) than those plants from the sheltered location (Santibáñez, both subtidal and intertidal) (post hoc multiple comparisons, $p < 0.05$). Leaves from the intertidal plants in Santibáñez were the stiffest, whereas those from the other locations had a similar elasticity (E_T, Fig. 4d). Seasonality in biomechanical traits was not so patent as spatial variation, although significant for some of them. Leaves were more extensible (L_T) and tougher (W_{TS}) during autumn and winter, except for those plants from El Bajo de la Cabezuela, which presented an unclear pattern (Fig. 3d, e). Leaves from subtidal plants in Santibáñez were more resistant (higher absolute force-to-tear, W_{TA}) during winter, compared to other seasons (Fig. 3a). The material stiffness (E_T) did not show any distinct seasonal pattern (Fig. 3f).

Spatial and seasonal variation in fibre content

Leaf fibre content varied very little (10 %), without seasonal variation but showing significant differences between locations (Table 2; Fig. 5). Higher amounts of fibre content were recorded in leaves from the most exposed location (El Chato), the medium exposed location (El Bajo de la

Fig. 3 Seasonal variation of mechanical leaf traits of *C. nodosa* per location. Mean values are represented with confident intervals (95 %). F_{TA}, absolute force-to-tear; F_{TS}, specific force-to-tear (tensile strength); W_{TA}, absolute work-to-tear; W_{TS}, specific work-to-tear (tensile toughness); L_T, elongation-to-tear (breaking strain); E_T, modulus of elasticity in tension (material stiffness). Locations (from high to low exposure): CH, El Chato (high exposure to waves, intertidal); BC, Bajo de la Cabezuela (medium exposure to waves and currents, subtidal); ST, Santibáñez (low exposure to waves and currents) with two stations: intertidal (ST-int) and subtidal (ST-sub)

Cabezuela), and from subtidal plants of the sheltered location (Santibáñez) in comparison with plants from intertidal Santibáñez (sheltered location) (Fig. 5).

Discussion

Despite the wealth of research in morphological plasticity of seagrass leaves, variation in leaf biomechanics and fibre content has been poorly reported, even though being crucial in plant ecological performance, especially regarding physical forces. We hypothesised that leaves of the temperate species *C. nodosa* may present plasticity in both morphology and biomechanical properties to accommodate the environmental heterogeneity of different abiotic factors, mainly hydrodynamics. In addition, we expected high leaf fibre content in leaves with high values of tensile strength. Firstly, we found that *C. nodosa* manifested variability in both leaf morphology and biomechanical traits due to spatial and seasonal environmental

Fig. 4 Seasonal variation of leaf fibre content of *C. nodosa* per location. Mean values are represented with confident intervals (95 %). Locations (from high to low exposure): CH, El Chato (high exposure to waves, intertidal); BC, Bajo de la Cabezuela (medium exposure to waves and currents, subtidal); ST, Santibáñez (low exposure to waves and currents) with two stations: intertidal (ST-int) and subtidal (ST-sub)
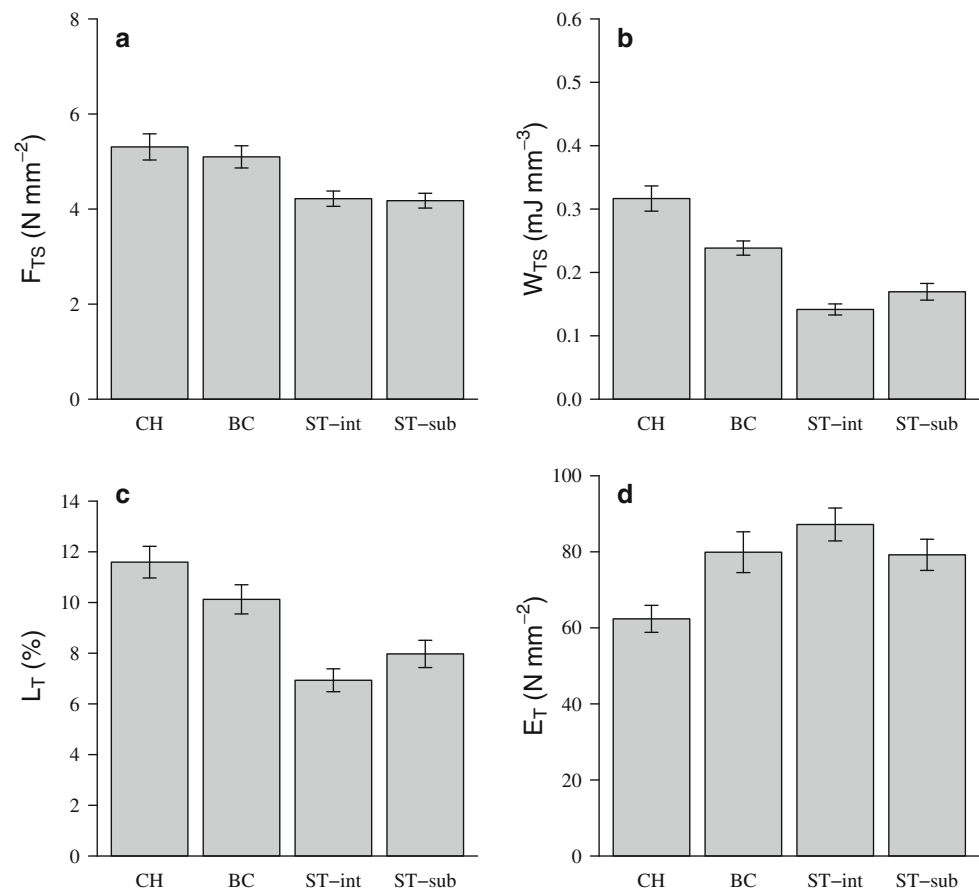
heterogeneity. Secondly, leaf fibre content displayed a low intraspecific variation, being invariant along the year and with little variation, yet significant, among locations, but not necessary correlated with the leaf tensile strength.
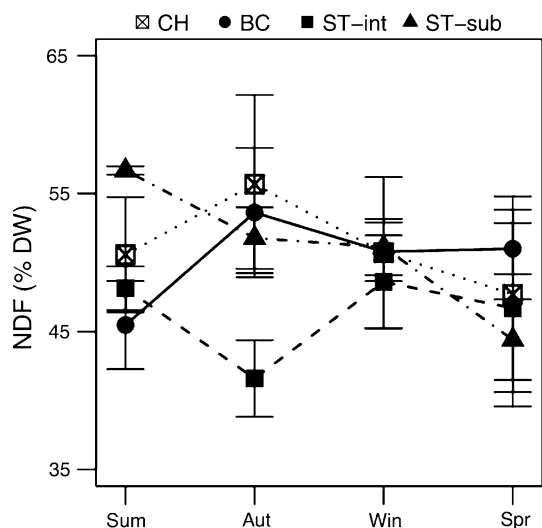
Variation in leaf morpho-mechanical traits

Environmental factors associated with depth and tidal position, mainly light availability and emersion duration, are probably the main factors responsible for the variation observed in leaf morphology. Light is the driving force in seagrass acclimation, leading to changes in the architectural features such as leaf length or cross-sectional area (Peralta et al. 2002; Olesen et al. 2002; Lee et al. 2007; de los Santos et al. 2010; Olivé et al. 2012). In Santibáñez, *C. nodosa* exhibits a strategy to cope with small depth differences, which includes an increase in leaf length to increase light use efficiency (Olivé et al., 2012; Pérez-Lloréns et al. unpublished). In addition, tidal position is probably affecting the leaf length of intertidal plants from Santibáñez, since decreasing it may be beneficial against desiccation in the intertidal zone (e.g. Cabaço et al. 2009). The reduced size of plants from the intertidal pools in El Chato, which are not emerged during low tide, is likely to be explained by the high hydrodynamic conditions they

face, since a dwarfed morphotype is the most common response to mechanical stress in aquatic plants (Puijalon and Bornette 2004; Peralta et al. 2005, 2006), being advantageous through drag reduction (Bouma et al. 2005).

Whole-leaf mechanical properties, such as absolute force-to-tear (F_{TA}), determine the mechanical resistance and depend on the distribution of the material (i.e. cross-sectional area) and the material properties (i.e. material strength). Consequently, any variation in leaf morphology (especially cross-sectional area) due to environmental acclimation entails changes in the mechanical resistance. In our study, only subtle differences in leaf material properties, but great differences in mechanical resistance, were found among locations. Thus, we suggest that distribution of the material (i.e. cross-sectional area), rather than changes in their make-up (i.e. material properties or leaf fibre content), determines the resistance of *C. nodosa* leaves to mechanical forces, as found for aquatic plants (Etnier and Villani 2007). This concept explains the major resistance in plants from subtidal locations (Santibáñez and Bajo de la Cabezuela), where plants acclimates to light reduction by increasing their leaf length and cross-sectional area (Olivé et al. 2012), thereby increasing their mechanical resistance as a *byproduct*, even if they are not exposed to high physical loads. Kopp (1999) found that *Z. marina*

Fig. 5 Variation of mechanical leaf traits of *C. nodosa* per location. Mean values are represented with confident intervals (95 %). F_{TA}, absolute force-to-tear; F_{TS}, specific force-to-tear (tensile strength); W_{TA}, absolute work-to-tear; W_{TS}, specific work-to-tear (tensile toughness); L_T, elongation-to-tear (breaking strain); E_T, modulus of elasticity in tension (material stiffness). Locations (from *high to low* exposure): CH, El Chato (high exposure to waves, intertidal); BC, Bajo de la Cabezuela (medium exposure to waves and currents, subtidal); ST, Santibáñez (low exposure to waves and currents) with two stations: intertidal (ST-int) and subtidal (ST-sub)

leaves resisted higher tensile forces during the active growing season than during wintertime. This is in agreement with our results for *C. nodosa*, as the absolute force-to-tear was higher in spring and summer (growing seasons) than in winter. The higher mechanical resistance during the growing seasons may be explained by the increase in cross-sectional area, since neither the fibre content nor the F_{TS} increase during that period.

Although the most noticeable variation was shown for the absolute mechanical resistance, variation was also found in material properties (size-independent). We suggest that high hydrodynamic conditions account for the tough and extensible leaves of wave-exposed *C. nodosa* thriving in the intertidal pools of the wave-swept shore in El Chato beach. High leaf toughness and extensibility make plants good energy absorbers, what may increase their hydrodynamical performance under wave-exposed environments. In fact, sessile marine organisms have several way of being tough (Koehl 1982): by being extensible (high L_T) although weak (low F_{TA}), or by being stiff (high E_T) although not very extensible (low L_T). Our observations in wave-exposed *C. nodosa* plants point to the importance of seagrass toughness and extensibility, as well as a reduced size, in facing high mechanical loads despite leaves being weak (low F_{TA}). Although we point to hydrodynamic as the main factor to explain the found variability, other environmental variables may account for

the variation in the leaf material properties. For instance, leaf stiffness and tensile strength of *Z. noltii* (La Nafie et al. 2012) and two aquatic plant species (Lamberti-Raverot and Puijalon 2012) were reduced under nutrient enrichment in mesocosm experiments.

Leaf fibre content

Changes in leaf biomechanical properties reflect underlying variations in cellular structures, possibly via cell orientation, proportion of strengthened cell wall materials and/or leaf anatomy (Biehle et al. 1998; Onoda et al. 2004; Lamberti-Raverot and Puijalon 2012). For instance, differences in the biomechanical properties along hydrodynamic gradients are often attributed to variations in the amount and/or composition of the cell walls in macroalgae, although there is little evidence of intraspecific variation (reviewed in Hurd 2000). Accordingly, we found that the intraspecific variation in fibre content was very low. Similar studies conducted on freshwater plants found a greater proportion of strengthened cell wall material in plants collected from fast-flowing environments in comparison with the same species from slow current ones (Biehle et al. 1998). We obtained that plants from the most exposed location (El Chato beach) presented slightly higher amount of fibres than individuals from other locations. However, plants from subtidal Santibáñez, where mechanical forces are not pronounced, also presented a high amount of fibre, what may be necessary to account for the increase in size that they experienced in the subtidal zone. Therefore, acclimation to environmental heterogeneity by changing morpho-mechanical properties of the leaves may be costly in terms of supporting tissue, thus likely entailing a trade-off in resource allocation and growth investment.

Conclusions

We reported a new aspect in seagrass leaf plasticity under environmental heterogeneity: leaf mechanical properties varied seasonally and spatially in temperate species *C. nodosa*. Different abiotic factors may be influencing the morpho-biomechanical variations reported. On the whole, leaf mechanical resistance may be a byproduct of acclimation to any environmental factor which entails changes in leaf morphology, for instance, light or nutrients. However, we hypothesise that hydrodynamics may be one of the main factors responsible for the spatial patterns in material properties (size-independent). We concluded that leaf mechanical resistance of seagrasses depends to a larger extent on the distribution of the material (i.e. cross-sectional area) rather than on material properties or fibre content at intraspecific level. Seagrass capacity to thrive

under a range of mechanical forces may be dictated by their plasticity in morpho-biomechanical traits, a key element for the hydrodynamical performance. This variation may result in differential fitness and may be an important trait, facilitating occurrence in some habitats, hence explaining species colonisation and distribution.

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ERRATUM

Erratum to: New aspect in seagrass acclimation: leaf mechanical properties vary spatially and seasonally in the temperate species *Cymodocea nodosa* Ucria (Ascherson)

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Unfortunately, in the original version of the article, figure captions 4 and 5 were interchanged. Additionally, in figure 4 caption, two variables were unnecessarily included. The corrected captions along with their Figs. 4 and 5 are given in the following page:
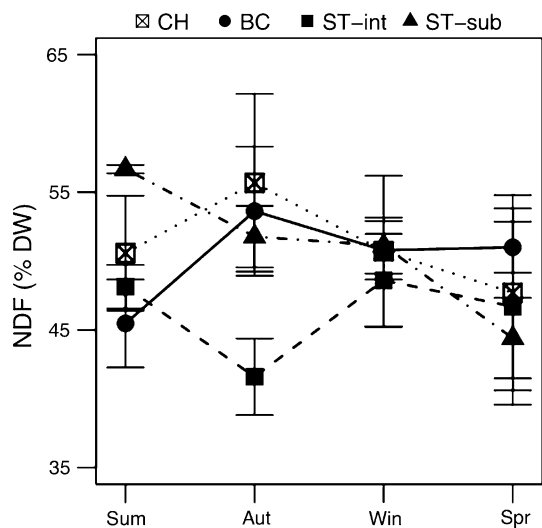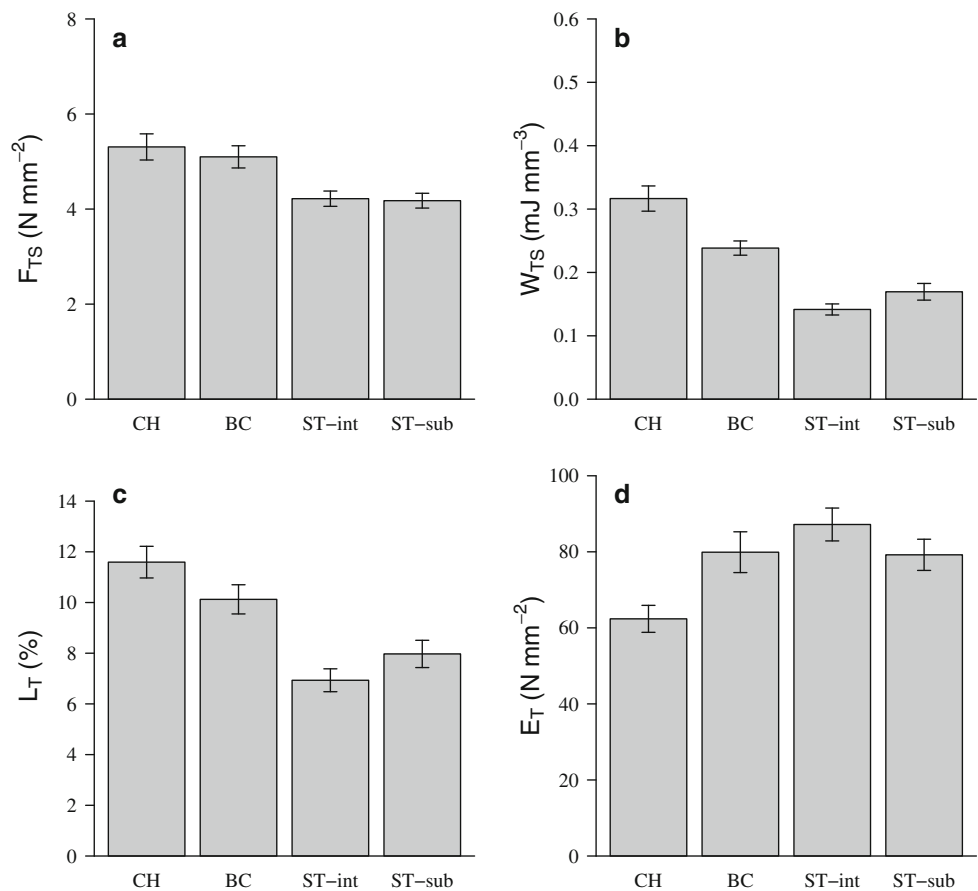

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Fig. 4 Variation of mechanical leaf traits of *C. nodosa* per location. Mean values are represented with confident intervals (95 %). F_{TS}, specific force-to-tear (tensile strength); W_{TS}, specific work-to-tear (tensile toughness); L_T, elongation-to-tear (breaking strain); E_T, modulus of elasticity in tension (material stiffness). Locations (from high to low exposure): CH, El Chato (high exposure to waves, intertidal); BC, Bajo de la Cabezuela (medium exposure to waves and currents, subtidal); ST, Santibáñez (low exposure to waves and currents) with two stations: intertidal (ST-int) and subtidal (ST-sub)

Fig. 5 Seasonal variation of leaf fibre content of *C. nodosa* per location. Mean values are represented with confident intervals (95 %). Locations (from high to low exposure): CH, El Chato (high exposure to waves, intertidal); BC, Bajo de la Cabezuela (medium exposure to waves and currents, subtidal); ST, Santibáñez (low exposure to waves and currents) with two stations: intertidal (ST-int) and subtidal (ST-sub)