

1 **The association of leaf sulfur content with the leaf economics spectrum and plant** 2 **adaptive strategies**

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14

15 **Abstract**

16 Sulfur is an essential macronutrient for plant primary metabolism, and its availability can modulate
17 plant growth in most terrestrial ecosystems. Despite this, its relationship with other leaf and nutrient
18 traits, and hence its contribution to plant functioning, remains unclear.

19 We analysed leaf and nutrient traits for 740 vascular plant species growing in a wide range of
20 environmental conditions in Northern Italy. We aimed to determine whether leaf sulfur content per
21 unit leaf dry mass (LSC) is associated with the leaf economics spectrum, and whether its distribution
22 among functional types (growth forms, leaf life span categories, and Grime's CSR (Competitive,
23 Stress-tolerant, Ruderal) strategies) could help to elucidate adaptive differences within plant taxa.

24 High LSC values were mainly associated with fast-growing species representative of R- and C-
25 strategy selection, thus the acquisitive extreme of plant economics, reflecting strong potential
26 connections with ecosystem properties such as biomass production or litter decomposability. In
27 general, LSC was significantly and positively correlated with leaf nitrogen content, and nitrogen to

28 sulfur ratio was constant throughout growth forms, leaf life span and CSR strategies, and phylogenetic
29 effects were evident.

30 Our findings highlight that LSC variation is strongly associated with the leaf economics spectrum,
31 suggesting that additional nutrients seldom included in functional analyses may also be embroiled
32 within the context of plant economics. However, different ratios among nitrogen and sulfur may be
33 expected across different plant families, suggesting that deeper insight from functional groups can
34 provide a bridge between plant stoichiometry and ecology, useful for the evaluation of ecological
35 responses to global change.

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37 **Key words**

38 Plant functional traits, global spectrum, Grime's strategies, CSR, leaf nutrients, ecological
39 stoichiometry

40

41 **Author contribution**

42 **Michele Dalle Fratte**: Conceptualization, Methodology, Investigation, Visualization, Writing-
43 Original draft preparation, Writing- Reviewing and Editing. **Simon Pierce**: Writing- Reviewing and
44 Editing. **Magda Zanzottera**: Investigation, Writing- Reviewing and Editing. **Bruno E.L.**
45 **Cerabolini**: Conceptualization, Methodology, Writing- Reviewing and Editing, Supervision.

46 **1. Introduction**

47 Sulfur is one of the macronutrients essential for plant growth and physiological functions and is
48 vital for metabolic processes (Aerts and Chapin 1999, Marschner 2012). Sulfur availability has a
49 particularly important effect on photosynthesis because it is a component of the proteins involved in
50 chlorophyll synthesis (Hu et al. 2017) and chloroplast formation (Hasanuzzaman et al. 2018).
51 Together with nitrogen, sulfur is a component of essential organic compounds, such as proteins
52 (amino acids cysteine and methionine), vitamins (biotin and thiamine), cofactors (Co-A and S-
53 adenosyl methionine) and various other secondary compounds (Marschner 2012). Moreover, sulfur
54 protects plants from biotic (i.e. herbivory; Badenes-Perez et al. 2014) and abiotic stresses such as
55 salinity, drought, and toxic metals/metalloids (Hasanuzzaman et al. 2018), through different
56 compounds that directly act as antioxidants or modulate antioxidant defence systems (e.g. glutathione,
57 phytochelatins, metallothioneins) (Hawkesford and De Kok 2007). Despite the relevance of sulfur for
58 plant metabolism, few studies have investigated its stoichiometry in terrestrial ecosystems (Han et al.
59 2011, Legay et al. 2014, Miatto and Batalha 2016, Sardans et al. 2016, Shi et al. 2016, Wu et al.
60 2017), while much attention has been paid to the stoichiometry among carbon, nitrogen and
61 phosphorus, due to their greater contribution to plant productivity.

62 Under natural conditions, the major sulfur sources for plants are soil sulfates that originate from
63 the mineralization of organic matter. However, plants may acquire, through the leaves, other forms
64 of sulfur for growth, e.g. smaller quantities of sulfur dioxide (SO₂; De Kok et al. 2017) from
65 deposition following either natural (volcanic activity or sulfur springs) or anthropogenic (industrial)
66 emissions (Bussotti et al. 2005). Until the 1980s, the amount of sulfur fertilization increased along
67 with SO₂ emissions due to industrial activity, but simultaneously SO₂ was the main contributor to the
68 significant intensification of acid deposition such that sulfur was mainly regarded as a pollutant.
69 National and international legislation on emission-reductions lead to the current worldwide trend of
70 decreasing SO₂ emissions (Aas et al. 2019), and to a sulfur deficiency scenario at mid latitudes of the

71 northern hemisphere. Sulfur limitation decreases growth of wild species and yields of crops
72 (Hawkesford and De Kok 2007), but the extent to which it could become a limiting element is
73 unknown (Johnson et al. 2018). In order to understand and predict the effects of sulfur limitation, the
74 resource economics of this nutrient (i.e. how plants manage and allocate sulfur between different
75 functions) must be determined.

76 Leaf nutrient contents of terrestrial plants are typically related to environmental factors (see Dalle
77 Fratte et al. 2019), the uptake and assimilation of nutrients should reflect a trade-off between
78 metabolic requirements of plants and nutrient availability, in turn reflecting natural selection and thus
79 species adaptation. The demand for sulfur is highly dependent on the availability of nutrients coupled
80 with environmental conditions (Hawkesford and De Kok 2007), and varies strongly between species,
81 also in relation to taxonomy (Zhang et al. 2012, Wu et al. 2017, De La Riva et al. 2018). Among leaf
82 nutrient contents, nitrogen has been identified as the main representative of global scale trade-offs
83 between resource capture and conservation (Díaz et al. 2016). Such variation in plant resource
84 allocation has been extensively investigated within the context of the leaf economics spectrum (LES;
85 Wright et al. 2004, Reich 2014). Rapid resource acquisition is usually correlated with high values of
86 specific leaf area (SLA) or leaf nitrogen content (LNC), while high leaf dry matter content (LDMC)
87 or leaf carbon content (LCC), lignin content or carbon to nitrogen ratio (C/N) reflect a resource
88 conservation strategy (Pierce et al. 2007, Freschet et al. 2010, De La Riva et al. 2018).

89 Hence, it is expected that leaf sulfur content per unit leaf dry mass (hereafter LSC) is correlated
90 with traits related to the LES and is embroiled in the balance between acquisitive and conservative
91 species. However, interactions between LSC and other plant traits have been studied only locally
92 and/or for only a few species (e.g. Bussotti et al. 2005, Sardans et al. 2008, Laliberté et al. 2012). De
93 La Riva et al. (2018) highlighted that the use of functional groups (leaf life span and habitat type)
94 appears to be useful to untangle the relationship between LSC and plant functioning. Consequently,
95 we could expect a strong relation among LSC and functional groups related to the LES, such as
96 growth form or leaf life span (Wright et al. 2004). Grime's CSR (Competitive, Stress-tolerant,

97 Ruderal) adaptive strategies scheme (Grime 2006) also defines functional groups identified on a solid
98 theoretical basis and is the only ecological strategy theory that simultaneously explains both
99 economics and size as fundamental gradients of plant adaptation and evolution (Grime and Pierce
100 2012). Considering that the extremes of the LES roughly correspond to stress-tolerant (conservative)
101 and ruderal (acquisitive) CSR strategies (Díaz et al. 2016), these should also be connected to LSC.
102 Although it is evident that low nitrogen and phosphorus contents are typically related to stress-tolerant
103 strategies (see Güsewell 2004 and references therein), we are not aware of comparative studies of
104 LSC in relation to CSR strategies.

105 Here, we sampled a large dataset of the vascular flora of Northern Italy, representative of a wide
106 range of environmental conditions of mid latitudes of the northern hemisphere, and tested whether:
107 1) LSC varies in concert with major axes of plant adaptation, in particular with the LES, 2) LSC
108 distribution is related to specific growth forms, leaf life span categories and plant families along the
109 LES, 3) LSC relates in different ways to CSR adaptive strategies.

110 **2. Material and methods**

111 **2.1. Dataset**

112 Our dataset (LIFTH, Leaf and nutrient Italian Flora Traits Hoard; Table S1) consists of 740 species
113 belonging to 99 families, of which only 17 are represented by at least 10 species records (Table S2).

114 The dataset includes leaf and nutrient traits of both wild species characteristic of the main habitats of
115 Northern Italy, and domesticated species (i.e. only cultivated in gardens and public parks).

116 Nomenclature of each taxon (family, genus, species) in our dataset was standardized according to
117 The Plant List (TPL, www.theplantlist.org) using the R package ‘Taxonstand’ (Cayuela et al. 2017).

118 For each species, within the same population we sampled from 5 to 15 fully expanded leaves
119 selected randomly from the outer canopy of different individual adult plants (at least 5 individuals)
120 growing in nature. All sampled populations were located far from possible anthropogenic SO₂
121 emission sources, enough to suppose that there was no soil sulfur excess due to S deposition, also
122 considering the high mobility of sulfate in soils (Johnson et al. 2018) in response to the negative
123 trends of SO₂ deposition observed over all European countries in the last two decades (EEA 2020).

124 For domesticated species, individuals were selected only in the middle of very large urban parks and
125 far from roads. Only for rare domesticated trees (e.g. *Sequoiadendron giganteum*) for which only a

126 single individual was present at a specific site, we sampled leaves from the outer canopy of the same
127 individual. Species were sampled at the peak of their vegetative growth over three years, from 2017

128 to 2019. Sampling sites were widespread over an area of approximately 50,000 km² ranging between
129 43°16' – 46°34' N latitudes and 07°54' – 11°00' E longitudes, with an altitudinal range of 2760

130 meters (30 - 2790 meters a.s.l.), i.e. from sea coasts to higher mountain belts. Indeed, even though
131 most of the species in our dataset are representative of vegetation types of the Alpine (Southern Alps)

132 and the Continental (Po Plain) biogeographical regions, an appreciable number of samples (7.3 %)
133 were collected within the Mediterranean region. Consequently, climate regimes, geological substrates

134 and soils of the sampling sites show extensive variability.

135 Laboratory measurements followed a standardized methodological protocol (Perez-Harguindeguy
136 et al. 2016): we stored leaf material at 4°C overnight to obtain full turgidity for the determination of
137 leaf fresh weight (LFW) and leaf area (LA; i.e. the surface area of fully expanded leaves); petioles
138 and rachides were included as part of the leaf. LA was determined using a digital scanner and the
139 software Leaf Area Measurement (LAM v.1.3; University of Sheffield, UK). Leaf dry weight (LDW)
140 was then determined after drying for 24 h at 105 °C, and SLA was then calculated as the ratio between
141 LA and LDW. For each species we computed the mean value of all plant traits and we also derived
142 adult plant height (H; i.e. maximum height above ground level of photosynthetic leaf tissue along the
143 main vertical axis of the individual) from literature data (Pignatti 1982). The dry leaf material was
144 then mixed and ground, and three randomly selected replicates were processed with a CHNS-analyzer
145 (FlashEA 1112 series Thermo-Scientific), obtaining values of LCC, LHC (Leaf Hydrogen Content),
146 LNC and LSC per leaf dry mass. For each of the three replicates, we also calculated the ratio between
147 LCC and LNC as well as LSC (respectively C/N and C/S ratios), and the ratio between LNC and LSC
148 (N/S ratio), and averaged values for each species, before transforming with a natural logarithmic
149 function [$\log(x)$]. Finally, using LA, SLA and LDMC, we calculated the C-, S-, and R- scores,
150 according to the *StrateFy* tool of Pierce et al. (2017) and then classified species into seven categories
151 including primary and secondary strategies (Grime 2006): C (Competitive), CR (Competitive –
152 Ruderal), R (Ruderal), SR (Stress-tolerant – Ruderal), S (Stress-tolerant), SC (Stress-tolerant –
153 Competitive), CSR (C – S – R strategist). The *StrateFy* tool (Pierce et al. 2017) compares the trade-
154 offs between LA, SLA and LDMC for each target species against worldwide variation in these traits,
155 allowing the extent of acquisitive vs. conservative leaf economics and plant size to be quantified and
156 compared against absolute global limits. This method has been shown to represent variability in
157 fourteen whole-plant, flowering, seed, and leaf functional traits (Pierce et al. 2017) and has the
158 advantage of using trait variation evident amongst vascular plants in general, including woody and
159 herbaceous species and taxa such as ferns, and can thus be applied to large datasets of wild plant
160 species and their communities.

161 We categorised species into woody (n = 204, including trees, shrubs and lianas), and non-woody (n
162 = 536, either long-lived or short-lived herbs), but also according to their leaf life span, which plays a
163 critical role in plant economics (Reich 2014). We adopted three categories: evergreen (n = 64),
164 wintergreen (n = 308), deciduous (n = 368). Accordingly, evergreen are those plants having leaves or
165 needles all year round and older than one year, while deciduous have leaves that die off after having
166 been green during summer, and only rarely some leaves may remain green during mild winters.
167 Wintergreen species are those plants that develop leaves during the vegetation period, but remain
168 green until the next leaf unfurling in spring unless they are exposed to very low temperatures or
169 extreme drought.

170 **2.2. Statistical analysis**

171 We computed all the statistical analyses using R software (R Core Team 2020). We performed a
172 principal component analysis (PCA) on standardized data, i.e. scaled to zero mean and unit variance,
173 using the function 'PCA' of the package 'FactoMineR' (Lê et al. 2008), to highlight the main adaptive
174 trends within the trait space of our dataset and to seek relationships of LSC with this. We achieved
175 this analysis using five plant traits (H, LA, LCC, LNC, SLA) that represent the major axes of plant
176 adaptation worldwide (Díaz et al. 2016), to which we added LSC. Before running the PCA, data were
177 first checked for normality by means of the Shapiro-Wilk test and accordingly we transformed all
178 variables using a natural logarithmic function [$\log(x)$]. The number of significant dimensions were
179 identified by comparing the eigenvalues of each component with the value given by the broken stick
180 distribution, and statistically significant correlations between PCA axes and plant traits were
181 identified using Pearson's correlation coefficient.

182 The following analyses were run on raw data of the same traits used in the PCA, as well as on
183 nutrient ratios (C/N, C/S and N/S). We applied a two-way mixed effect aligned ranks transformation
184 analysis of variance (ART ANOVA) to find differences of plant traits among growth form, leaf life
185 span categories, and their interaction term, using the function 'art' of the package 'ARTool' (Kay and

186 Wobbrok 2016); to exclude influences of phylogenetic correlation we considered the family as a
187 random effect. We also applied one-way ART ANOVA to test differences of plant traits among CSR
188 strategy categories as well as plant families, considering only those represented by at least 10 species
189 (Table S2). For each variable, we built a linear model from aligned and ranked data using the function
190 ‘artlm’, from which we computed least squares means through the function ‘emmeans’ of the package
191 ‘emmeans’ (Lenth et al. 2018) that we used to run pairwise post-hoc comparisons adjusted by the
192 Bonferroni correction method.

193 We used simple linear regression models to evaluate the log-log relationship of LSC (dependent
194 variable) with LNC (independent variables) within the whole dataset, as well as within each single
195 category of growth form, leaf life span, CSR plant strategies, and within each plant family
196 (represented by at least 10 species). The log-log regression has been shown to provide effective
197 insights concerning nitrogen and sulfur acquisition (Legay et al. 2014; Wu et al. 2017) and, in order
198 to provide further evidence of this relationship, we also ran the same linear regression models based
199 on the raw data. We considered the R^2 to assess the amount of variation explained by each regression
200 model. We used the base R package ‘stats’ for simple linear regression, and the package ‘ggtern’
201 (Hamilton and Ferry 2018) for the ternary visualization of CSR strategies.

202 3. Results

203 The average (\pm standard deviation) LSC in all the dataset was 2.1 ± 1.5 mg/g, and values spanned
204 a range of 11.3 mg/g, from a minimum of 0.3 to a maximum of 11.6 mg/g. The highest value was
205 measured for *Cardamine asarifolia*, and the lowest for both *Cupressus macrocarpa* and *Hieracium*
206 *amplexicaule* (Table 1 and Table S1).

207 The main pattern of species in the PCA were represented by a two-dimensional space (cumulative
208 percentage of variance = 64 %, Fig. 1 and Fig. S1) defined by variation in the LES (PC1 = 40 %) and
209 of the size and the dimensions of leaves and plant height (PC2 = 24%). The first axis (PC1) correlated
210 negatively with LCC, and positively with SLA and LNC (Table 2), indicating variation in trait values
211 ranging from conservative strategies to values indicating acquisitive resource use, while PC2
212 correlated positively with H and LA (i.e. towards taller plants with larger leaves). LSC correlated
213 significantly and positively with PC1; high values of LSC grouped with trait values indicating
214 acquisitive strategies, opposed to low values associated to the conservative extreme.

215 The LES was thus well defined by 4 traits, including LSC, which also showed statistically
216 significant differences among growth form categories (Fig. 2 and Table S3). In general, woody
217 species exhibited the lowest mean values of all the traits indicating acquisitive strategies (SLA, LNC
218 and LSC), opposed to non-woody species which displayed the highest mean values. However, among
219 leaf life span categories, this difference was significant only within deciduous species, for SLA and
220 LNC, and within wintergreen species, for LSC. Regarding LCC, woody species showed the highest
221 mean values, for evergreen and deciduous species, but not for wintergreen species. Concerning leaf
222 life span categories, evergreen species were more conservative compared to deciduous and
223 wintergreen, which were indeed more acquisitive, but this trend was significant only for woody
224 species, for SLA and LCC. Among non-woody species, evergreen showed lower values compared to
225 deciduous species, for LNC, and to wintergreen, for LSC. Among nutrient ratios (Fig. 4a-c and Table
226 S3), both C/N and C/S mirrored the same pattern of LES traits, showing higher values for woody

227 species, specifically evergreen species, while N/S ratio was stable among all growth form and leaf
228 life span categories.

229 Ruderal (R) and competitive (C) species, as well as species with both competitive and ruderal
230 characteristics (CR), showed on average the highest LSC values, in contrast to S-selected species,
231 which exhibited the lowest values, only comparable to those of the SC category (Fig. 3b). Only a few
232 species, among those with higher LSC, were ordinated towards the centre of the CSR triangle
233 (*Erysimum rhaeticum* and *Mercurialis annua*) or even towards the S-selected corner (*Equisetum*
234 *fluviatile*) (Fig. 3a). Accordingly, stress-tolerant (S) species, and those with both stress-tolerant and
235 competitive characteristics (SC), showed the highest mean values of carbon to nutrient ratios (both
236 C/N and C/S) (Fig. 4d-f and Table S3), while N/S ratio did not exhibit significant differences among
237 CSR strategy categories.

238 Only a few plant families exhibited significant differences of the mean values of traits correlated
239 with the LES (Fig. S2). The *Pinaceae* family was found to be the most conservative, while
240 *Brassicaceae* and *Caryophyllaceae* were the most acquisitive, despite not showing significant
241 differences compared to many families with intermediate characteristics. Traits involved in the LES
242 confirmed *Pinaceae* and *Brassicaceae* to be families that were significantly different from others,
243 respectively placed at the conservative and acquisitive extremes, even though *Brassicaceae* showed
244 the highest mean values only for nutrient traits, while for SLA, the highest mean value was that of
245 *Caryophyllaceae*. Carbon to nutrient ratios showed a similar pattern, even though C/N discriminated
246 more the *Pinaceae*, while C/S the *Brassicaceae*. Considering the average values of N/S ratio, the
247 *Brassicaceae* showed the lowest value, but the trend for all the other families was not much coherent
248 with the LES, with higher values exhibited by *Leguminosae*, *Orchidaceae*, and *Rosaceae*, despite the
249 latter being one of the most conservative families.

250 LSC showed a significant positive linear relationship with LNC (Table S4) considering all species
251 together (slope = 0.81, $R^2 = 0.30$, $p < 0.01$), which was even more robust within wintergreen species
252 (slope = 0.93, $R^2 = 0.32$, $p < 0.01$) and within the R strategy category (slope = 0.95, $R^2 = 0.42$, $p <$

253 0.01). Regarding families, we found an increase of the R^2 compared to the overall equation for
254 *Asparagaceae*, *Caprifoliaceae*, *Caryophyllaceae*, *Juncaceae*, *Orchidaceae*, *Poaceae*, and *Rosaceae*.

255 4. Discussion

256 LSC of the study species was in the range published for the leaves of both crops and wild species
257 from different terrestrial ecosystems (e.g. Dijkshoorn and Van Wijk 1997, Bussotti et al. 2005, Han
258 et al. 2011, Zhang et al. 2012, Wu et al. 2017, De La Riva et al. 2018). Wu et al. (2017) studied LSC
259 in the forests of China and, despite determining wide variation according to forest types, they found
260 an average value of 1.1 mg/g, which is lower than that of our dataset (2.1 mg/g), likely because they
261 sampled only woody species, most of which belonged to *Gymnospermae*. Furthermore, their dataset
262 was obtained from published literature and referred to samples collected before the increasing trend
263 of SO₂ emissions in China since the 1980s. Indeed, a comparable study (Han et al. 2011) reported a
264 mean value of 1.58 mg/g, which is similar to the value of 1.6 mg/g that we found for woody species
265 in our study. In contrast, crop species can have higher variation of LSC, and values for sulfur deficient
266 plants can drop to lower than 0.1 mg/g (Dijkshoorn and Van Wijk 1997), which was not reached in
267 our study. Our findings suggest that the decreasing trend of sulfur emissions over the last two decades
268 in Europe may still have not led to sulfur limitations.

269 Our analysis at the regional scale confirmed the robustness of the two main axes of variation of
270 plant functioning (Fig. 1 and Table 2): representing the LES (i.e. fast-slow leaf economics; Reich
271 2014), and the size and dimensions of plant and leaves (both comprising the global spectrum of plant
272 form and function; Díaz et al. 2016). The pattern of SLA, LCC, LNC, together with LSC, reflected,
273 the acquisitive-to-conservative trade-off determining the LES (Wright et al. 2004, Reich 2014),
274 demonstrating that LSC variation was coupled with the LES, supporting Hypothesis 1. Compared to
275 the global spectrum, in our study the LES was the dimension accounting for the largest source of
276 variance. This leads to the main finding of our work, namely that LSC is evidently associated with
277 LES variation, suggesting that additional nutrients seldom included in functional analyses may also
278 be embroiled in the LES (Sardans et al. 2008, Laliberté et al. 2012, De La Riva et al. 2018). Indeed,
279 plant growth requires at least 17 mineral elements used in leaves to support fundamental physiological

280 processes (Marschner 2012) and leaf nutrient contents are supposedly closely related to SLA across
281 species (Wright and Cannon 2001, Wright et al. 2004), likely because most elements end up in the
282 cytoplasm rather than cell walls, which are indeed thinner for 'acquisitive' species. Moreover, the
283 LES is fundamentally a trade-off between investment in structural polymers and metabolic
284 machinery, which performance is guaranteed by sulfur and other mineral nutrients that are essential
285 components of proteins.

286 Nitrogen together with phosphorus are integral to proteins of the photosynthetic apparatus,
287 including Rubisco, and their contents are thus positively correlated with net CO₂ assimilation rate,
288 dark respiration rate and relative growth rate (Güsewell 2004, Reich et al. 2008). We also observed a
289 significant linear relationship between LSC and LNC (Table S4) that confirmed previous similar
290 results (Legay et al. 2014, Wu et al. 2017, Dijkshoorn and Van Wijk 1997), highlighting that the
291 assimilation processes of sulfur and nitrogen follow similar patterns (Hasanuzzaman et al. 2018), as
292 most available reduced nitrogen and sulfur are incorporated into amino acids and subsequently into
293 proteins (De Kok et al. 2017). Sulfur has specific roles in fundamental processes, besides chloroplast
294 formation, from photosynthesis to carbon and nitrogen metabolism (Droux 2004), including roles not
295 shared by nitrogen and phosphorus but relevant to key biochemical pathways. For example, it
296 contributes to ferredoxin oxidation (De Kok et al. 2017), and is part of Iron-sulfur clusters that aid
297 the production of protein sufB, required for chlorophyll production (Hu et al. 2017). Sulfur thus
298 represents a co-limitation to chlorophyll content and photosynthetic capacity with respect to nitrogen
299 contents (Resurreccion et al. 2001) and is vital to the achievement of the higher photosynthetic and
300 respiration rates typical of acquisitive species (Wright et al. 2004).

301 In contrast, conservative species exhibit greater mechanical support (e.g. high LCC) and low
302 photosynthetic and respiration rates, which takes longer to pay back the leaf construction costs (Villar
303 and Merino 2001). C/N and C/S provide an indication of the relative investment in structure (carbon)
304 and cell functioning (nitrogen and sulfur) and thus represent alternative measures of resource
305 economics with respect to SLA or LDMC (Freschet et al. 2010), which use just mass measurements.

306 Species having leaves with a higher C/N are usually slow growing (Pierce et al. 2007, Freschet et al.
307 2010) with higher lignin content, and this optimization has also been found to relate to defence against
308 herbivory (Hanley et al. 2007). Thus, the addition of C/S to this framework could aid predictions
309 concerning plant resistance to herbivory, as it expresses not only leaf digestibility (which corresponds
310 with high leaf nutrient contents) but it also informs with regard to plant toxicity provided by sulfur-
311 based plant defence metabolites (e.g. Badenes-Perez et al. 2014). Moreover, leaf C/N has often been
312 used as a proxy of environmental variables such as soil organic matter quality and litter decomposition
313 rates (Freschet et al. 2010, Rosenfield et al. 2020). Based on our findings, it seems reasonable to
314 extend the same interpretation of C/N also to C/S, in terms of carbon dynamics and nutrient economy
315 at the community level (Laliberté et al. 2012, Bruelheide et al. 2018).

316 Plant functional types have often been invoked to explain differences existing along the LES
317 (Reich 2014, Díaz et al. 2016), indeed we detected significant differences classifying species into
318 both growth form and leaf life span categories, confirming our second hypothesis. Woody species, as
319 well as evergreen species, showed higher affinities for conservative strategies, in contrast to non-
320 woody, as well as wintergreen and deciduous species, which overall demonstrated relatively
321 acquisitive strategies. This interpretation was supported by differences in mean values of all analysed
322 leaf and nutrient traits, including sulfur traits (Fig. 2 and Fig. 4). Evergreen species, compared to
323 wintergreen and deciduous, have tougher leaves, so that their higher value of LCC and
324 correspondingly lower SLA are related to a greater portion of carbon in structural tissue (Villar and
325 Merino 2001), which makes them less susceptible to environmental hazards and stress (Bussotti et al.
326 2005, Sardans et al. 2008, Poorter et al. 2009). They also displayed lower LNC and LSC, associated
327 with higher C/N and C/S, indicating low nutrient requirements due to a more effective use, typical of
328 slow growing species (Sardans et al. 2016, De La Riva et al. 2018), despite the greater investment in
329 tissue construction (Villar and Merino 2001). We observed similar large differences in traits related
330 to the LES among woody and non-woody growth forms, showing once more that the use of growth
331 forms can help to discriminate variations along the gradients underlying the LES (Wright et al. 2004)

332 and nutrient use efficiency, as herbaceous species are those with higher leaf nutrient contents (Han et
333 al. 2011), sulfur included. Nevertheless, at the global scale woody and herbaceous species have shown
334 extensive overlap along the LES (Díaz et al. 2016), partially due to the larger number of species and
335 range of climates that were considered in the global study. However, we found only herbaceous
336 species at the most acquisitive extreme of the LES, given their adaptation to include relatively
337 acquisitive traits (Pierce et al. 2013), as opposed to woody species that have a more conservative set
338 of traits.

339 Relating to our third hypothesis, we found evidence that high levels of LSC strongly relate to
340 ruderal and competitive strategies (the extent of R- and C-selection), while the stress tolerant strategy
341 (S-selection) was characterized by the lowest LSC values, confirming that Grime's CSR adaptive
342 strategies reflect global spectrum axes also with regard to nutrient economics (Güsewell 2004, Pierce
343 et al. 2007) (Fig. 3). Only a few species with a high LSC were located towards the stress-tolerant (S-
344 selection) corner; specifically, *Equisetum fluviatile*. This is not surprising if we consider that
345 *Equisetaceae* are known to have both high nutrient contents and a large accumulation of silica bodies.
346 The high nutrient contents of horsetails is due to an efficient nutrient uptake that allows these species
347 to thrive under a wide range of conditions (Husby 2013). At the same time, they are the best known
348 biosilicifer plants since they exhibit an abnormally high accumulation of silica, up to 25 % of dry
349 weight (Gierlinger et al. 2008) that, combined with the development of photosynthetic stems, may
350 provide heavier structures and hence greater affinities to stress-tolerant strategies.

351 Leaf nutrient contents are also linked to taxonomy (Zhang et al. 2012, Miatto and Batalha 2016),
352 as we clearly observed for the *Pinaceae* (and other *Gymnospermae*) and for the *Brassicaceae*,
353 respectively placed at the conservative and acquisitive extremes of the LES (Fig. 1 and Fig. S2).
354 *Brassicaceae* displayed the highest values of LSC, probably because of the production of sulfur-
355 containing plant resistance compounds, such as glucosinolates (Dijkshoorn and Van Wijk 1997,
356 Badenes-Perez et al. 2014). They also showed high values of LNC, which was loosely but
357 significantly correlated with LSC ($R^2 = 0.20$, $p < 0.05$; Table S4). A tighter linear relationship

358 between LSC and LNC (i.e. higher values of R^2 ; Table S4) was instead shown by other families as
359 linear regressions highlighted consistent differences. Furthermore, the N/S ratio largely varied among
360 families, with *Brassicaceae* and *Leguminosae* showing respectively the lowest and the highest values
361 of N/S ratio. However, N/S was constant across growth forms, leaf life span and CSR strategies
362 allowing the prediction of LSC from LNC and other leaf traits, at least at the extremes of the LES.
363 Our findings suggest that a broad understanding of sulfur economics can largely rely on functional
364 groups, rather than on families, because of marked taxonomic differences of primary and secondary
365 metabolic pathways.

366 We observed high relevance of sulfur for plant functioning that can be integrated with other plant
367 traits to learn more about the processes and patterns of ecosystem development in response to
368 environmental changes. Future changes of nutrient deposition loads, together with climate change,
369 will determine new environmental scenarios that may substantially alter the chemical composition of
370 terrestrial ecosystems, with profound consequences for competition among species, plant community
371 composition and biogeochemical cycles (Shi et al. 2016). Ultimately, the biological stoichiometry of
372 plants can play a key role in exploring evolutionary processes and adaptive variation in the biota, thus
373 is crucial in evaluating ecological responses to global change (Elser et al. 2010). However, few LSC
374 data appear to be available today in international trait databases (e.g. in TRY, Kattge et al. 2020),
375 compared to other leaf nutrient contents, particularly LNC. In light of this, here we presented a large
376 dataset of leaf traits, including LNC and LSC as relevant, covering a wide range of plant forms and
377 functions, useful to integrate trait-based analysis, at least at the regional or national scale (Chelli et
378 al. 2019).

379 **5. Conclusions**

380 The link identified here between sulfur traits, LSC and C/S, and other leaf traits underlying the
381 LES provides insights concerning the role of sulfur for plant functioning. Specifically, we found that
382 species with high LSC were associated with the acquisitive extreme of the LES detected at the global
383 scale, which was represented by highly ruderal (R) and competitive (C) strategies. The relationships
384 between sulfur and other nutrients, specifically LCC, LNC and C/N, suggests that sulfur traits also
385 scale up to ecosystem properties, such as biomass production or litter decomposability, which are
386 strictly related with LES traits.

387

388 **Acknowledgements**

389 This work was funded by Fondazione Lombardia per l'Ambiente (FLA). We thank Andrea
390 Gianotti, Matteo Francocci and Martina Guglielmi for assistance in the field and in the laboratory.

391

392 **Conflicts of interest**

393 The authors declare that they have no conflict of interest.

394

395 **Data availability statement**

396 All data supporting the analysis are available in supplementary materials (Table S1) and, on
397 acceptance, they will be deposited at TRY Plant Trait Database <https://try-db.org/TryWeb/Home.php>

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520 **Tables**

521 **Table 1:** Descriptive statistics of LSC (leaf sulfur content per leaf dry mass) for all species, growth
 522 form, leaf life span and CSR plant strategy categories. Legend: n = number of species, min =
 523 minimum, max = maximum, st.dev. = standard deviation, 25th and 75th are respectively the first and
 524 third quantiles, while 10th and 90th are respectively the first and ninth deciles.

525

		mean	st.dev	min	10 th	25 th	median	75 th	90 th	max	n
ALL	-	2.1	1.5	0.3	0.9	1.2	1.7	2.5	3.8	11.6	740
Growth form	woody	1.6	0.9	0.3	0.8	1.0	1.5	1.9	2.7	5.2	204
	non-woody	2.3	1.6	0.3	0.9	1.3	1.8	2.7	4.0	11.6	536
Leaf life span	deciduous	2.2	1.4	0.6	1.0	1.3	1.8	2.5	3.7	9.3	368
	wintergreen	2.3	1.7	0.3	0.9	1.3	1.7	2.7	4.1	11.6	308
	evergreen	1.2	0.7	0.3	0.6	0.7	1.0	1.4	1.8	3.9	64
Growth form : Leaf life span	woody-deciduous	1.9	1.0	0.6	1.0	1.2	1.6	2.3	3.2	5.2	121
	woody-wintergreen	1.6	0.5	0.8	1.0	1.1	1.5	2.0	2.2	2.6	24
	woody-evergreen	1.2	0.7	0.3	0.6	0.7	1.0	1.4	1.9	3.9	59
	non-woody-deciduous	2.3	1.5	0.7	1.1	1.4	1.9	2.6	3.9	9.3	247
	non-woody-wintergreen	2.3	1.7	0.3	0.9	1.3	1.7	2.8	4.3	11.6	284
	non-woody-evergreen	1.1	0.4	0.5	0.8	1.1	1.2	1.2	1.4	1.5	5
CSR categories	C	2.9	2.2	0.8	1.2	1.5	2.2	3.2	5.8	11.6	44
	CR	2.7	1.8	0.3	1.3	1.5	2.1	3.2	5.1	11.0	128
	CSR	2.2	1.3	0.8	1.1	1.4	1.8	2.4	3.5	10.0	93
	R	2.5	1.5	0.7	1.4	1.5	2.1	2.9	4.4	8.2	69
	S	1.5	1.2	0.3	0.7	0.9	1.2	1.7	2.4	10.7	144
	SC	1.8	1.1	0.4	0.9	1.1	1.6	1.9	3.2	5.9	143
	SR	2.1	1.3	0.5	0.9	1.3	1.7	2.6	3.6	8.6	119

526

527 **Table 2:** Pearson's correlation coefficients (r) of the values of traits involved in the principal
528 component analysis (PCA), C-, S- and R- scores, and the first two components (PC1 and PC2) of the
529 PCA, for the 740 plant species investigated; strong correlations (r between ± 0.50 and ± 1) are
530 emboldened. Legend: H = plant height, LA = leaf area, LCC = leaf carbon content, LNC = leaf
531 nitrogen content, LSC = leaf sulfur content, SLA = specific leaf area, ns = not significant, * = p-value
532 < 0.05 , ** = p-value < 0.01 , *** = p-value < 0.001 .

533

	LA	LCC	LNC	LSC	SLA	C	S	R	PC1	PC2
H	0.34 ***	0.37 ***	-0.08 *	-0.09 *	-0.39 ***	0.24 ***	0.23 ***	-0.52 ***	-0.42 ***	0.78 ***
LA		-0.1 **	0.29 ***	0.14 ***	0.09 *	0.92 ***	-0.40 ***	-0.18 ***	0.22 ***	0.79 ***
LCC			-0.21 ***	-0.39 ***	-0.38 ***	-0.16 ***	0.42 ***	-0.37 ***	-0.65 ***	0.23 ***
LNC				0.55 ***	0.56 ***	0.33 ***	-0.57 ***	0.48 ***	0.77 ***	0.34 ***
LSC					0.37 ***	0.19 ***	-0.39 ***	0.35 ***	0.73 ***	0.20 ***
SLA						0.09 *	-0.70 ***	0.86 ***	0.80 ***	-0.12 ***
C							-0.56 ***	-0.07 *	0.28 ***	0.69 ***
S								-0.72 ***	-0.72 ***	-0.15 ***
R									0.72 **	-0.35 ***

534

535 **Figure captions**

536 **Figure 1:** Principal Component Analysis on traits values of the 740 vascular plant species
537 investigated. Species points are coloured by RGB classification according to their CSR scores
538 calculated with *StrateFy* (Pierce et al. 2017): red = C, green = S, blue = R. Labelled points are species
539 with the three highest and lowest values on PC1 and PC2. Legend: H = plant height, LA = leaf area,
540 SLA = specific leaf area, LCC, LNC and LSC = respectively leaf carbon, nitrogen and sulfur content
541 per leaf dry mass..

542

543 **Figure 2:** Boxplots of traits involved in the LES (Leaf Economics Spectrum) of woody (black) and
544 non-woody (grey) species for each leaf life span category, displaying the mean (diamond), median
545 (line in the middle of the boxes), the interquartile range (boxes), ± 1.5 times the interquartile range
546 (whiskers) and outliers (circle). Results of the aligned ranks transformation ANOVAs are reported in
547 Table S3; small letters represent pairwise post-hoc comparisons at $p < 0.01$. Legend: SLA = specific
548 leaf area, LCC, LNC and LSC = respectively leaf carbon, nitrogen and sulfur content per leaf dry
549 mass..

550

551 **Figure 3:** (a) Ternary visualization of CSR (Competitive, Stress-tolerant, Ruderal) strategies of the
552 740 species selected for the analysis; darker and wider points denote higher values of LSC (leaf sulfur
553 content per leaf dry mass). (b) Boxplots of LSC among plant strategy categories displaying the mean
554 (diamond), median (line in the middle of the boxes), the interquartile range (boxes), ± 1.5 times the
555 interquartile range (whiskers) and outliers (circle) ($F = 13.9$, $p < 0.0001$); small letters represent
556 pairwise post-hoc comparison.

557

558 **Figure 4:** Boxplots of nutrient ratios based on leaf dry mass of woody (black) and non-woody (grey)
559 species for each leaf life span category (a, b, c), and among plant strategy categories (d, e, f),

560 displaying the mean (diamond), median (line in the middle of the boxes), the interquartile range
561 (boxes), ± 1.5 times the interquartile range (whiskers) and outliers (circle). Results of the aligned rank
562 transformation ANOVAs are reported in Table S3; small letters represent pairwise post-hoc
563 comparisons at $p < 0.01$. Legend: C/N = carbon to nitrogen, C/S = carbon to sulfur, N/S = nitrogen
564 to sulfur. All nutrient ratios are reported in natural logarithmic scale.