

1 **Nitrogen status regulates morphological adaptation of marsh plants to elevated CO₂**

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9 **Coastal wetlands provide valuable ecosystem services that are increasingly threatened by**
10 **anthropogenic activities¹. Atmospheric carbon dioxide (CO₂) concentration has increased**
11 **from 280 to 404 ppm since the industrial revolution, and is projected to exceed 900 ppm by**
12 **2100². In terrestrial ecosystems, elevated CO₂ typically stimulates C₃ plant photosynthesis**
13 **and primary productivity leading to an increase in plant size³. However, compared to woody**
14 **plants or crops⁴, the morphological responses of clonal non-woody plants to elevated CO₂**
15 **have been less well-studied. We show that 30 years of experimental CO₂ enrichment in a**
16 **brackish marsh increased primary productivity and stem density, but decreased the stem**
17 **diameter and height of the dominant clonal species *Schoenoplectus americanus*. Smaller,**
18 **denser stems were associated with the expansion of roots and rhizomes to alleviate nitrogen**
19 **(N) limitation as evidenced by high N immobilization in live tissue and litter, high tissue C:N,**
20 **and low available porewater N. Changes in morphology and tissue chemistry induced by**
21 **elevated CO₂ were reversed by N addition. We demonstrate that morphological responses to**
22 **CO₂ and N supply in a clonal plant species influences the capacity of marshes to gain elevation**
23 **at rates that keep pace with rising sea levels.**

24 Terrestrial plants are experiencing the highest atmospheric CO₂ concentration in the past
25 800,000 years⁵. The stimulation of carbon (C) fixation by elevated CO₂ -- the CO₂ fertilization
26 effect -- is well documented⁶. Increases in leaf-level C uptake rates are often accompanied by
27 changes in plant morphology or morphometric sizes, such as increased height, stem diameter,
28 leaf area index, leaf number, and root volume^{7, 8, 9, 10}. These morphometric changes are widely
29 observed to influence competitive dynamics^{11, 12}, with implications for ecosystem structure and
30 function.

31 Resource availability regulates the effect of elevated CO₂ on total biomass production but
32 can also influence changes in plant size and patterns of biomass allocation. Elevated CO₂-
33 induced changes in plant morphology are poorly understood compared to changes in total
34 biomass, and morphological changes are highly variable along environmental gradients and
35 between plant functional groups, reflecting species-specific strategies for resource acquisition^{7,}
36 ⁹. For instance, plant species can overcome N limitation caused by CO₂ fertilization in low-N
37 environments if they possess traits to mine and recycle N through shared underground
38 networks of ectomycorrhizae¹³, illustrating the importance of understanding how resource
39 acquisition influences plant responses to elevated CO₂. Similarly, clonal plants employ shared
40 underground networks of roots and rhizomes to exploit heterogeneous soil resources, affording
41 a competitive advantage in resource-poor environments¹⁴. Clonal plants are ubiquitous,
42 occurring in 10 out of 11 classes of vascular plants, and are an important component of many
43 ecosystems¹⁵; yet, their morphological responses to elevated CO₂ are understudied compared
44 to other functional groups such as non-clonal trees, shrubs, herbs, and crops. Clonal
45 architecture has important implications for biomass allocation and resource acquisition strategy

46 that can propagate into ecosystem level responses to global change. For example, growth
47 allocation to rhizomes and ramets may influence stem density and decrease interannual
48 variation in plant growth by storing resources when conditions are favorable and remobilizing
49 them when conditions are adverse¹⁴.

50 Our objective was to examine the morphological responses of a clonal marsh plant species
51 to elevated CO₂ and N addition using long-term data from two field experiments in a tidal
52 marsh on the Chesapeake Bay. The native plant community is dominated by the perennial C₃
53 sedge *Schoenoplectus americanus* and two co-dominant C₄ grasses, *Spartina patens* and
54 *Distichlis spicata*. *Experiment 1* began in 1987 and consists of open-top chambers ventilated
55 with either ambient air or CO₂ concentrations elevated to 700-800 ppm. *Experiment 2* began in
56 2006 to investigate interactions between elevated CO₂ and N addition with 4 treatments:
57 ambient CO₂, ambient CO₂ + N, elevated CO₂, and elevated CO₂ + N. In both experiments, we
58 made annual measurements of stem morphology (height, width, and density), stem biomass,
59 belowground productivity, tissue chemistry, porewater ammonium (NH₄⁺, started in 2002 in
60 *Experiment 1*), and soil surface elevation change. Morphometric data were collected only for *S.*
61 *americanus*; the morphometric responses of C₄ grasses were not examined because the growth
62 form of these species does not lend itself to such measurements¹⁶.

63 Elevated CO₂ increased sedge total biomass by an average of 20% over the control in
64 *Experiment 1*. The CO₂-driven increase in belowground biomass productivity (34% ± 7) was
65 larger than the increase in aboveground biomass (17% ± 4), resulting in a 16% ± 6 increase in
66 the below:above biomass ratio (Fig. 1a). Simultaneously, the density of stems increased 51% ± 6

67 (Fig. 2a, Appendix 1, Table S1) and the biomass of individual sedge stems decreased $16\% \pm 1$
68 (Fig. 2b), corresponding to a 5% decline in stem height and a 10% decline in diameter (Fig. 2c,
69 d). Moreover, stem density and rhizome biomass were positively correlated ($R^2=0.30$, $P <$
70 0.0001 , Fig. S1), suggesting that the CO_2 -induced increase in belowground allocation was
71 expressed through the clonal architecture of the sedge.

72 Plants shift biomass allocation between roots and shoots to optimize resource capture and
73 use, with allocation to photosynthetic aboveground biomass for CO_2 uptake, and to
74 belowground biomass for nutrient uptake¹⁷. Salt and brackish systems tend to be N limited
75 because the phosphorus that would be bound to iron in freshwater systems is liberated by the
76 sulfates in seawater¹⁸. N limitation is known to constrain the CO_2 fertilization effect^{13, 19, 20}, and
77 in our experiments elevated CO_2 induced the classic symptoms of progressive N limitation in
78 this coastal wetland ecosystem¹⁹. Elevated CO_2 plots in *Experiment 1* had more N sequestered
79 in plant biomass (i.e., 5% increase in shoot N and 12% increase in root N) and litter (21%
80 increment, Fig. 1a), and less plant-available inorganic soil N (i.e., 47% lower porewater $[\text{NH}_4^+]$;
81 $[\text{NO}_3^-]$ was below detection, Fig. 1a, Fig. S2a), implying soil N depletion in the rooting zone.
82 Elevated CO_2 increased the C:N ratio in sedge shoot, root, and rhizome biomass by 18%, 10%,
83 and 23%, respectively (Fig. 1a), consistent with a CO_2 -induced increase in N use efficiency²¹.
84 Preferential allocation of photosynthate to roots and the rhizome network helps alleviate N
85 depletion by expanding the surface area for N acquisition²². In this clonal species, rhizome
86 extension leads to increased tiller recruitment and ultimately higher stem density²³.

87 *Experiment 2* provides an experimental test of inferences about CO₂-induced N limitation
88 from *Experiment 1*. In *Experiment 2*, elevated CO₂ alone produced the same response in sedge
89 density and size observed *Experiment 1*, but the effect was absent when elevated CO₂ was
90 crossed with N addition, in which case individual *S. americanus* stem size increased (Fig. 1b). As
91 in *Experiment 1*, the CO₂-only treatment decreased inorganic N by 48% ± 4 (Fig. 1b, Fig. S2b),
92 while adding N to elevated CO₂ plots increased porewater inorganic N by 5% ± 9 higher than
93 that in ambient plots. Moreover, the N-only treatment resulted in a 15% decrease in
94 belowground productivity, indicating reduced plants biomass allocation to root systems for N
95 uptake under N enrichment.

96 In contrast to the common result that plant size increases under elevated CO₂, we
97 observed that elevated CO₂ caused a clonal plant to produce smaller individual stems at higher
98 density. While “shrinking stems” in response to elevated CO₂ have been observed in other C₃
99 grasses⁸, we clearly demonstrate that this is an indirect response to N deficiency in a clonal
100 plant species through manipulative experiments. We propose a conceptual framework for the
101 responses of clonal plant growth to CO₂ enrichment (Fig. 3). Elevated CO₂-stimulation of plant
102 productivity (clonal and non-clonal) leads to soil N deficiency because the increase in N demand
103 is not satisfied by a combination of increased N uptake and shifts in plant C:N ratio. In most
104 plant functional groups, this shift leads to an increase in growth allocation to belowground
105 structures and increased N uptake without necessarily changing plant density. However, in
106 clonal species, increasing belowground growth generates a more extensive rhizome system,
107 more tiller-recruitment of stems, and increased stem density. Because the increase in stem
108 density is not entirely compensated by higher ecosystem NPP, individual plant stems are

109 smaller. Because shrinking stems coincide with N depletion and can be reversed by N
110 enrichment (Fig. 1b), we conclude that the response of clonal plant morphology to elevated CO₂
111 is regulated by soil N limitation (Fig. 3, Fig. S3) and suggest that shrinking stem sizes can be used
112 as evidence of N limitation in clonal plant communities. Such responses to CO₂-induced N
113 limitation are observed in a variety of terrestrial ecosystems where clonal species occur, but the
114 consequences of allocation shifts for plant morphology, stand structure, and ecosystem
115 function have been largely overlooked.

116 The phenotypic plasticity of marsh plants in response to elevated CO₂ has an important
117 impact on the survival of coastal wetlands and their ecosystem services. The effectiveness of
118 wetland vegetation in dissipating storm energy is tied to the density and morphology of stems,
119 and their effect on frontal area (i.e., the total cross-sectional area of stems perpendicular to
120 flow velocity)^{24, 25}. We calculated changes in frontal area in response to elevated CO₂ in
121 *Experiment 1* and *2* and found significant increases in frontal area due to increased stem
122 density (Table 1). The analysis indicates that elevated CO₂ may enhance the value of marshes as
123 natural infrastructure for coastal protection through a mechanism tied to clonal traits²⁴. These
124 morphological changes in response to elevated CO₂ also have important implications for the
125 habitats of wetland birds, nekton and benthic invertebrates^{26, 27, 28}, and they influence key
126 ecosystem processes such as soil formation, C storage, and nutrient retention²⁹.

127 Perhaps most importantly, changes in root and shoot morphology directly influence the
128 capacity of coastal wetlands to build elevation in response to rising sea level^{30, 31}. At our high
129 marsh site in the Chesapeake Bay, 69% of the species are clonal, and clonal plants occupy 68%

130 of marsh area. Tradeoffs between shoot and root productivity under elevated CO₂ potentially
131 alter the balance between the contributions of surface mineral sedimentation and subsurface
132 root expansion to elevation gain¹. Long-term field measurements indicate that elevated CO₂-
133 enhanced belowground production increased elevation gain by 1.5 mm yr⁻¹ via subsurface
134 expansion³². As sea level rises and plants are flooded more frequently, aboveground biomass
135 and stem morphology also influence elevation gain by enhancing the settling of suspended
136 mineral sediments on the marsh surface^{33, 34, 35}. We explored the effects of changes in stem
137 structure on potential mineral deposition using a previously published model^{35, 36} to simulate
138 increased flooding 25 years in the future (~8 cm of increased flooding, Appendix 2). Modeled
139 surface sedimentation was enhanced an additional 0.7-1.5 mm yr⁻¹ by elevated CO₂ and the
140 combination of elevated CO₂ and N (Table 1), suggesting an aboveground mechanism for
141 increasing the resilience of C₃ marsh ecosystems to sea level rise. Feedbacks between
142 aboveground biomass and mineral sedimentation become stronger with increasing flooding
143 duration, and suspended sediment supply^{1, 35}, suggesting that the positive effects of elevated
144 CO₂ on elevation gain may amplify under these conditions. Such responses will vary with factors
145 such as suspended sediment supply, watershed N loading, plant traits, and other site-specific
146 characteristics. For example, plants responded to the combination of elevated CO₂ and N in
147 *Experiment 2* with an increase in stem size, density, aboveground biomass, and belowground
148 productivity compared to the ambient treatment, which translated into both higher modeled
149 surface accretion and subsurface expansion (Table 1). However, N can also cause root biomass
150 to decline³⁷, in which case elevation gains from increased surface accretion may be offset by
151 lower subsurface expansion. Process-based models informed by observations and experiments

152 are required to integrate the complex feedbacks that ultimately govern elevation gain. Plant
153 traits such as clonal architecture that govern density, height, root allocation, and other
154 morphological responses to global change have important ecosystem consequences that are
155 presently missing from forecast models.

156 We showed that elevated CO₂ and N addition elicit significant changes in the structure and
157 function of coastal marshes that arise from tractable plant traits such as clonal architecture.
158 The 30-year data set presented here raises additional questions that are outside the scope of
159 the present study. Firstly, Rasse et al. summarized the data from *Experiment 1* after 17 years
160 and found that *S. americanus* density in the C₃ community increased by 128% compared to 51%
161 in the present study³⁸ (Fig. 2a). Our results demonstrate that the direction of the density
162 responses remained the same after an additional 13 years CO₂ enrichment, but the amount of
163 stimulation has declined due to resource limitation, interactions with other variables such as
164 sea level rise, or other factors. Secondly, the stem biomass and density responses in the C₃
165 community changed over time (Table S1), perhaps with changes in salinity, NH₄⁺ discharges
166 from the Rhode River watershed, or other factors that require additional exploration (Table S2).
167 Thus, future studies must consider additional important factors such as warming temperatures,
168 changing precipitation and hydrologic extremes, changing salinity and inundation regimes, and
169 invasive species.

170

171 Supplementary Information

172 Appendix 1 and Appendix 2

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183 References

- 184 1. Kirwan, M. L. & Megonigal, J. P. Tidal wetland stability in the face of human impacts and
185 sea-level rise. *Nature* **504**, 53-60 (2013)
- 186 2. Collins, M., R. Knutti, J. Arblaster, J.-L. Dufresne, T. Fichet, P. Friedlingstein, X. Gao,
187 W.J. Gutowski, T. Johns, G. Krinner, M. Shongwe, C. Tebaldi, A.J. Weaver and M.
188 Wehner. Long-term Climate Change: Projections, Commitments and Irreversibility. *In:*
189 *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the*
190 *Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker,
191 T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and
192 P.M. Midgley (eds.)]. (2013)
- 193 3. Curtis, P. S. & Wang, X. Z. A meta-analysis of elevated CO₂ effects on woody plant mass,
194 form, and physiology. *Oecologia* **113**, 299-313 (1998)
- 195 4. Long, S. P. *et al.* Food for thought: lower-than-expected crop yield stimulation with
196 rising CO₂ concentrations. *Science* **312**, 1918-1920 (2006)
- 197 5. Luethi, D. *et al.* High-resolution carbon dioxide concentration record 650,000-800,000
198 years before present. *Nature* **453**, 379-382 (2008)
- 199 6. Ainsworth, E. A. & Rogers, A. The response of photosynthesis and stomatal conductance
200 to rising CO₂: mechanisms and environmental interactions. *Plant Cell and Environment*
201 **30**, 258-270 (2007)
- 202 7. Pritchard, S. G. *et al.* Elevated CO₂ and plant structure: a review. *Global Change Biology*
203 **5**, 807-837 (1999)
- 204 8. Wand, S. J. E. *et al.* Responses of wild C₄ and C₃ grass (Poaceae) species to elevated
205 atmospheric CO₂ concentration: a meta-analytic test of current theories and
206 perceptions. *Global Change Biology* **5**, 723-741 (1999)

- 207 9. Ainsworth, E. A. & Long, S. P. What have we learned from 15 years of free-air CO₂
208 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy.
209 *New Phytologist* **165**, 351-371 (2005)
- 210 10. Yang, L. X. *et al.* Seasonal changes in the effects of free-air CO₂ enrichment (FACE) on
211 growth, morphology and physiology of rice root at three levels of nitrogen fertilization.
212 *Global Change Biology* **14**, 1844-1853 (2008)
- 213 11. Schwinning, S. & Weiner, J. Mechanisms determining the degree of size asymmetry in
214 competition among plants. *Oecologia* **113**, 447-455 (1998)
- 215 12. White, K. P. *et al.* C₃ and C₄ biomass allocation responses to elevated CO₂ and nitrogen:
216 contrasting resource capture strategies. *Estuaries and Coasts* **35**, 1028-1035 (2012)
- 217 13. Terrer, C. *et al.* Mycorrhizal association as a primary control of the CO₂ fertilization
218 effect. *Science* **353**, 72-74 (2016)
- 219 14. Hutchings, M. J. & Dekroon, H. Foraging in plants: the role of morphological plasticity in
220 resource acquisition. *Advances in Ecological Research* **25**, 159-238 (1994)
- 221 15. Callaghan, T. V. *et al.* Clonal plants and environmental change: introduction to the
222 proceedings and summary. *Oikos* **63**, 341-347 (1992)
- 223 16. Lu, M. *et al.* Allometry data and equations for coastal marsh plants. *Ecology* **97**, 3554.
224 (2016)
- 225 17. Reynolds, H. L. & Pacala, S. W. An analytical treatment of root-to-shoot ratio and plant
226 competition for soil nutrient and light. *American Naturalist* **141**, 51-70 (1993)
- 227 18. Vitousek, P. M. & Howarth, R. W. Nitrogen limitation on land and in the sea: How can it
228 occur? *Biogeochemistry* **13**, 87-115 (1991)
- 229 19. Luo, Y. *et al.* Progressive nitrogen limitation of ecosystem responses to rising
230 atmospheric carbon dioxide. *Bioscience* **54**, 731-739 (2004)
- 231 20. Craine, J. M. *et al.* Isotopic evidence for oligotrophication of terrestrial ecosystems.
232 *Nature Ecology & Evolution* **2**, 1735-1744 (2018)
- 233 21. Niu, S. L. *et al.* Nitrogen regulation of the climate-carbon feedback: evidence from a
234 long-term global change experiment. *Ecology* **91**, 3261-3273 (2010)
- 235 22. Nie, M. *et al.* Altered root traits due to elevated CO₂: a meta-analysis. *Global Ecology*
236 *and Biogeography* **22**, 1095-1105 (2013)
- 237 23. Ikegami, M. Functional specialization of ramets in a clonal plant network. PhD thesis.
238 Utrecht University, Utrecht (2004)
- 239 24. Gedan, K. B. *et al.* The present and future role of coastal wetland vegetation in
240 protecting shorelines: answering recent challenges to the paradigm. *Climatic Change*
241 **106**, 7-29 (2011)
- 242 25. Sheng, Y. P. *et al.* The reduction of storm surge by vegetation canopies: Three-
243 dimensional simulations. *Geophysical Research Letters* **39**, L20601 (2012)
- 244 26. Vandolah, R. F. Factors regulating the distribution and population dynamics of the
245 amphipod *Gammarus palustris* in an intertidal salt marsh community. *Ecological*
246 *Monographs* **48**, 191-217 (1978)
- 247 27. Bertness, M. D. Fiddler crab regulation of *Spartina alterniflora* Production on a New
248 England salt marsh. *Ecology* **66**, 1042-1055 (1985)

- 249 28. Zedler, J. B. Canopy architecture of natural and planted cordgrass marshes: selecting
250 habitat evaluation criteria. *Ecological Applications* **3**, 123-138 (1993).
- 251 29. Drake, B. G. Rising sea level, temperature, and precipitation impact plant and ecosystem
252 responses to elevated CO₂ on a Chesapeake Bay wetland: review of a 28-year study.
253 *Global Change Biology* **20**, 3329-3343 (2014)
- 254 30. Nyman, J. A. *et al.* Marsh vertical accretion via vegetative growth. *Estuarine, Coastal and*
255 *Shelf Science* **69**, 370-380 (2006)
- 256 31. Cherry, J. A., McKee, K. L., Grace, J. B. Elevated CO₂ enhances biological contributions to
257 elevation change in coastal wetlands by offsetting stressors associated with sea-level
258 rise. *Journal of Ecology* **97**, 67-77 (2009)
- 259 32. Langley, J. A. *et al.* Elevated CO₂ stimulates marsh elevation gain, counterbalancing sea-
260 level rise. *Proceedings of the National Academy of Sciences of the United States of*
261 *America* **106**, 6182-6186 (2009)
- 262 33. Morris, J. T. *et al.* Responses of coastal wetlands to rising sea level. *Ecology* **83**, 2869-
263 2877 (2002)
- 264 34. Darke, A. K. & Megonigal, J. P. Control of sediment deposition rates in two mid-Atlantic
265 Coast tidal freshwater wetlands. *Estuarine Coastal and Shelf Science* **57**, 255-268 (2003)
- 266 35. Mudd, S. M. *et al.* How does vegetation affect sedimentation on tidal marshes?
267 Investigating particle capture and hydrodynamic controls on biologically mediated
268 sedimentation. *Journal of Geophysical Research-Earth Surface* **115**, F03029 (2010).
- 269 36. Kirwan, M. L. & Mudd, S. M. Response of salt-marsh carbon accumulation to climate
270 change. *Nature* **489**, 550-553 (2012).
- 271 37. Deegan, L. A. *et al.* Coastal eutrophication as a driver of salt marsh loss. *Nature* **490**, 388-393
272 (2012).
- 273 38. Rasse, D. P., Peresta G., Drake B. G. Seventeen years of elevated CO₂ exposure in a
274 Chesapeake Bay Wetland: sustained but contrasting responses of plant growth and CO₂
275 uptake. *Global Change Biology* **11**, 369-377 (2005)

276

277 **Author Contributions** The analysis was conceived by M.L. and J.P.M. The ongoing operation of the
278 experiments were conducted by J.P.M. and J.A.L. The data were compiled and analyzed by M.L.
279 Accretion modelling was performed by E.H. All authors contributed to writing the paper.

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281 authors declare no competing financial interests. Correspondence and requests for materials should be
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283 Methods

284 The open top chamber (OTC) CO₂ enrichment experiment, initiated in 1986, is located at
285 Kirkpatrick marsh (38°53'N, 76°33'W), a 23-ha brackish salt marsh on the Rhode River estuary in
286 Chesapeake Bay, on the eastern coast of the United States. This common Chesapeake Bay high
287 marsh ecosystem is dominated by a perennial C₃ sedge *Schoenoplectus americanus*, and two
288 co-occurring C₄ grasses *Spartina patens* and *Distichlis spicata*. Mean annual temperature is 14.1
289 °C and mean annual precipitation is 948 mm. Mean tidal range is approximately 40 cm, and the
290 marsh platform is about 18 cm (in the C₃ plots) and 40 cm (in the C₄ plots) above daily mean low
291 water level. Water level is higher than mean marsh elevation about 25% of the time. The soils
292 contain approximately 80% organic matter (i.e. peat) to a depth of 5 m. The morphometric data
293 used in our analysis was available only for C₃ sedges; morphometric data are not collected on C₄
294 grasses in these long-term experiments because of their small stem diameter and high density
295 (Appendix 1).

296 *Experiment 1:* Three plant communities were distinguished in the marsh, a C₃ community
297 dominated by *S. americanus*, a C₄ community consisting of *S. patens* and *D. spicata*, and a
298 mixed C₃/C₄ community. OTC was used to increase the CO₂ concentration around the plants in
299 each of the three communities. Ten circular chambers of 80 cm diameter and 100 cm height
300 were placed in each community. In five of the chambers, atmospheric [CO₂] was elevated to
301 340 μl CO₂ l⁻¹ above the ambient CO₂ concentration (about 340 μl CO₂ l⁻¹ at the beginning of the
302 study). The CO₂ concentration in the other five chambers was ventilated with ambient air as the
303 ambient treatment. To determine a possible OTC effect on plant growth, five outside chamber

304 control sites in each community were compared with the ambient CO₂ chambers. CO₂ exposure
305 began each year when the plants emerged in the spring and continued 24h a day through
306 autumn following total senescence. A survey of all plots was conducted in 1986 before initiation
307 of the treatments and showed no significant differences in plant biomass assigned to the three
308 treatments in each community.

309 *Experiment 2:* Twenty octagonal OTCs of 200 cm height and 150 cm diameter were established
310 adjacent to the site of *Experiment 1* in 2006. OTCs were randomly assigned to one of four
311 treatment groups (n=5): ambient CO₂, ambient CO₂ + N, elevated CO₂, and elevated CO₂ + N.
312 CO₂ treatment was consistent with *Experiment 1*. N was added by spraying NH₄Cl monthly from
313 May to September each year (total 25 g N m⁻² year⁻¹).

314 Total height, green height and half height diameter of each C₃ sedge stem were measured and
315 stem density was counted in the plots each year. The dry mass of individual stems was
316 determined using an allometric equation based on destructively harvested samples¹⁶, and the
317 resulting mean biomass was multiplied by the stem density to estimate aboveground biomass.
318 Belowground plant productivity was estimated each year by three root ingrowth cores in each
319 plot. Nine porewater wells were placed in each chamber with three depths: 15, 30 and 75 cm.
320 Porewater was sampled and NH₄⁺ concentration was analyzed approximately monthly during
321 the growing season. In these anaerobic marsh soils, porewater NO₃⁻ is typically below detection
322 limits and does not contribute substantially to soil available N.

323 In this study, we calculated the response ratio to reflect the response of plant and porewater N
324 to elevated CO₂. Response ratio is defined as the ratio of the mean value of a given variable in

325 the treatment group (elevated CO₂ or elevated CO₂ plus N addition) to that in the control
326 group. The ratio for each year was calculated from the means of replicate plots (generally n=5)
327 in each treatment. The annual ratios were then averaged to reflect the treatment effect across
328 the 30-year (*Experiment 1*) or 11 year (*Experiment 2*) record. Repeated measures using a mixed
329 effects model was used to test for significant differences in stem biomass, height, diameter,
330 and densities between the elevated CO₂ and ambient CO₂ chambers in SAS program (version
331 9.0). A discrete autoregressive correlation model was conducted to test time effects. The
332 environmental and climate impact was examined with correlations between C₃ sedge
333 aboveground biomass and annualized environmental factors using Pearson's r statistic. Figures
334 and linear regression results were conducted using SigmaPlot (version 10.0, SPSS Inc., Chicago,
335 IL, USA).

336 We modeled changes in potential mineral accretion using a previously published model of
337 marsh vertical accretion that takes into account the height, diameter, and density of marsh
338 vegetation^{35, 36}. We used treatment-specific relationships between aboveground biomass and
339 stem structure (density, diameter, and height) from *Experiments 1 and 2*. Because mineral
340 accretion rates at this high elevation marsh are presently limited by very infrequent surface
341 flooding, we modeled the effect of biomass and stem structure on mineral accretion rates for a
342 future marsh that is lower elevation and more frequently flooded due to accelerated sea level
343 rise. Specifically, model experiments used a lower elevation of Kirkpatrick Marsh (0.1 m above
344 MSL), which is approximately 0.08 m below the current elevation of *Experiments 1 and 2*, to
345 simulate an increase in inundation due to sea level rise (25 years at the current 3.4 mm yr⁻¹)
346 where surface accretion would be a significant contribution to vertical change. All other

347 parameters reflect current conditions (suspended sediment = 25 mg L⁻¹, 44 cm tidal amplitude)
348 or original model parameters used by Mudd et al. 2010³⁵. Finally, we calculated frontal area (λ)
349 as a proxy for the potential of vegetation to disperse storm energy:

350
$$\lambda = \frac{whn}{A}$$

351 where w and h are the plot-mean stem width and height, n is the number of stems per plot,
352 and A is the plot area.

353

354 Data Availability

355 Morphometric and derived biomass data from the experiments are posted on the Global
356 Change Research Wetland website (<http://serc.si.edu/gcrew/data>) and all data are available
357 from the corresponding authors upon request.