Dear Anonymous Reviewer,

We are grateful for the comments you provided, which have greatly improved this manuscript. We addressed each of your comments (blue italic text) and provide explanations of the changes we made.

Kindly,
Dulcinea Groff

** There is a lot of analysis into explaining the variation in the isotopes, how that's controlled by plant physiology, but not much discussion and explanation of how these isotope signals will be used to reconstruct paleoclimate especially in context of applying this to a peat core (through time). Providing a roadmap for how changes in d13C and d18O will be interpreted would be useful and weather this is qualitative or can it be pushed further to be quantitative?**

Peat-based reconstructions may be limited to identifying periods of warm/dry or cold/wet conditions that are more extreme than our observed seasonal variations (or more similar to them). For now, this proxy remains qualitative, but more work could be done to evaluate this proxy to assess its suitability for quantitative reconstructions (perhaps with leaf wax alkenones, though our preliminary data on hydrogen isotopes in precipitation suggests this may not be feasible). Resolving temperature and moisture signals independently would likely require growth chamber experiments.

**Solution:** We agree and added an expanded discussion on paleoclimate reconstructions (Lines 303-337) with a conceptual figure (Fig. 8) outlining what this proxy can reconstruct, including time-averaging and methods (i.e. analysis of bulk, individual leaves or roots).

**There is the suggestion that this is going to really help us understand climate dynamics, but then there is not discussion of how. Is this going to provide temperature or relative humidity or both (there is not clear indication of which and both are correlated with the isotopes) and how to you disentangle any changes in source water d18O through time?**

The oxygen and carbon isotopic signatures are positively correlated in our study, which represents a modern snapshot of conditions. Based on this modern calibration work, we recommend measuring C and O values together, and that these values would identify the timing of transitions between warmer and drier to colder and wetter conditions that correlate with major hemispheric drivers in climate.

We didn't find a relationship between d18O in precipitation and the d18O of leaf cellulose in our 1-year study. It is possible that because the d18O in leaf water is controlled by source water and humidity, any changes in humidity confound a direct relationship between source water d18O values and leaf water/cellulose d18O. It may also be possible that the variation in source area did not affect the d18O enough for us to detect a significant impact on the d18O of the leaf cellulose. Based on this work, the source area could influence leaf cellulose d18O value in peat records if source area changes were greater than what we observed.

**Solution:** Thank you for pointing out that this is unclear in the manuscript. We added a section to the Discussion (Line 303-337) that more clearly outlines how this proxy would work in practice, including the conceptual figure (Fig. 8), and clarification about the disentangling source water d18O through time.

** Some discussion of how to do this for paleoclimate also needs to focus on how this study shows nicely that the leaves are recording a seasonal signal. So, when you go down core, how are you going to deal with this? Are you going to focus on a large sampling of leaves from each horizon (age?) with the expectation that you are sampling both seasons or is it going to be a single multiple leaf measurement to approximate an annual signal? Some thought into this is needed as the data analysis and presentation may need to be added to or adapted for paleo work. I'd like to see a clearer connection between this nice modern calibration data and how to use it for the past.**

We recommend incorporating a large sampling of leaves from a horizon (1 cm), and expect that to be a sampling of both seasons, incorporating several years. These peat records are highly productive, but even so, time averaging within a given 1-cm level should be greater than than the age of a leaf. In the peat records we've analyzed, sedimentation rates can be ~20-30 years/cm, with most records extending at least 12,500 years old. As with any prehistoric reconstruction, it is important to consider temporal grain and resolution, which is going to constrain the inferences you can make.

**Solution**: Thank you for pointing out the need for this additional discussion. We included new text in the Discussion section (Lines 303-337) and conceptual figure (Fig. 8) with a recommended workflow and considerations for applying to peat records, including our recommendations for methods (e.g., including that multiple whole leaf fragments should be used from each level).

*Line 28: "trends in southern hemisphere climate dynamics" – is that consistent with what you can actually do with this proxy? Or is it something more specific?*

Based on what we have established with this study, this proxy can indicate trends in conditions similar to what we observed seasonally: warm/dry, cool/wet, which is more specific than our generalized statement. Multiple paleo-records could point to changes in Southern Hemisphere climate dynamics, but can not necessarily resolve the drivers of those dynamics.

*Lines 43-46: Awkward sentence with semicolon connecting two separate statements.*

Thanks for pointing this out. **Solution:** We removed the semicolon and edited for clarity (Lines 44-45).

*Line 56: Is it really called a "bog"? That's not confusing... It's hard to reconcile this description with the one line 70 and "pedestal" which is in the caption for Figure 2. Maybe some annotation on the figure or more description would be useful. I'd like to have a clear idea how this is going to develop over time in a peatland and how this plants growth habit is going to translate into a vertical succession (or some crazy patchwork of different ages in a peat core).*

Yes, colloquially each pedestal is called a "bog," and we removed this from the manuscript to avoid confusion -- especially because the tussac peat that forms the soils in these stands is not a bog, either. We called it a "pedestal" throughout. ("Tussac/tussock" is already confusing enough.)

The taphonomy of these pedestals is poorly understood. There could be a patchwork of ages within a peatland, but as of yet we have not found any evidence of age reversals or other chronology problems in our cores.

*Line 57: Something wrong with new sentence that starts here and sentence seems incomplete too.*

Thank you for pointing this out. **Solution:** We edited the sentence as "Smith and Prince (1985) established radiocarbon…" on Line 57.

*Line 56-57: Either they use precip or the precip wets all that organic matter and then there is evaporative enrichment b/c it is exposed to wind/sun.*

Fair point. **Solution:** We edited the text on Line 56 to indicate this.

*Line 70: Maybe start a new paragraph here or have a better transition?*

Thank you for this suggestion. We started a new paragraph and improved the opening sentence on Lines 70-73.

*Line 71 and below: check the order in which isotopes are first described. Here delta symbols are used first but aren't defined, next sentence doesn't use delta symbols (carbon isotopes), and then defined on line 90-91. I think this comes up a few other places and would be worth cleaning up.*

Thanks for noting this. We fixed this for consistency throughout.

*Lines 92-93: Improving "westerly wind dynamics" is different than what's mentioned elsewhere. What is it that this new proxy can solve and make it consistent throughout.*

Great point. We made the text clearer regarding exactly what climate conditions and questions this proxy can inform, while still placing it within the broader discussion of Southern Hemisphere climate dynamics. We used "Southern Hemisphere climate dynamics" on Line 93 and remained consistent throughout.

*Line 100: Could the km hr-1 also be reported here and later for reference? Not to many readers will think about wind speed in m/s.*

The International System of Units recommends m/s, so we will retain use of this standard for consistency with them, and with other studies (https://physics.nist.gov/cuu/Units/units.html). **Solution**: We added km/h in parenthesis after the recommended m/s (Lines 179, 181, and 183).

*Line 170-172: How are temperature and humidity related? Based on the figure, they look highly correlated. If they are, then how do you disentangle their effects from the cellulose d13C and d18O as they are both strongly related? I didn't see any multiple regression analysis reported below either.*

As discussed in the new section in the Discussion, we made this clearer (Lines 303-337). These variables are definitely correlated, and we do not think they can be disentangled further without additional measurements.

*Line 186: is west, NW, and SW 79%? That's missing from the sentence. Reporting 21% for the last source and not saying anything about the other 3 directions is reads strangely and compared to the prior sentence.*

Thank you for pointing out this missing reported value. **Solution:** This is now re-written on Lines 186-188: "In winter, 79% of the air mass back trajectories (n = 332 individual trajectories) were from the west, NW, and SW, while 21% of air masses had backward trajectories south of…".

*Line 206-207: I think you need to be really careful presenting this here and then in the discussion below. With this data, maybe the other factors have a stronger control than precipitation d18O, but at least at some level, precip d18O must be important. So, when applying this down core (through Holocene), if there are changes in d18O, they must change the cellulose d18O (and then it's probably modified by the other factors you report here). I think this is critical to point out for those who will use this in paleo applications. More on this below.*

We agree. **Solution:** We addressed this more clearly in the new section of the Discussion (Lines 303-337) and conceptual figure (Fig. 8) on paleo applications to make this clear. We rephrased the sentence using clearer language on Lines 208-209: "The δ18Oleaf or δ18Oroot did not correlate with δ18O in precipitation across all sites (Table 1)."

*Section 3.4: there's no mention here of the relationships between the isotopes and temp and humidity but these are in the discussion, figures and tables. This would be a good place to describe the relationships of to both environmental factors.*

This is addressed in section 3.5.

*Line 237: What negative correlation? Not in the results or the figures. VPD is not discussed prior to this.*

Thank you for identifying this mistake. It now reads "positive". **Solution:** We made the following changes on Line 239: "Ferrio and Voltas (2005) established a positive correlation between δ13Cleaf and vapor pressure deficit, suggesting stomatal conductance is sensitive to atmospheric moisture conditions."

*Line 242-244: Is this consistent with the "low" humidity of the Falklands of >70%?*

Yes, we changed this sentence to explain on Lines 245-247. **Solution:** "As plant stomata close in response to low humidity and/or high evaporative conditions like high wind speeds in the Falklands, the internal partial pressure of CO2 decreases and the δ13Cleaf increases (Farquhar et al., 1982, p.198)."

*Lines 283-288: Relating plant tissue d18O (or dD) to precipitation is always a challenge. Even if you had leaf water or soil (pedestal?) water, it would still be complicated, but maybe give some insight. Many studies try to relate d18O of the plant back to precipitation, but here, it's clear that other factors modify this. But, at the most basic level, d18O precip is setting source water and then maybe there is mixing with other sources (ground water, dew, etc), but that is then modified by temp/humidity, etc. I think some discussion here is needed to highlight that this is much more complicated than indicated for the reader. If one tries to do this down core, changes* *in d18Oprecip must at some level matter for the d18O of plant source water and ultimately the d18O cellulose.*

We agree. Thank you for pointing this out. **Solution:** We addressed this in the Discussion by adding sentences here to signal that this is not straightforward on Lines 303-337.

*Also, getting into event precip (as mentioned) could be interesting, but it might be more informative to pull into this discussion when the leaves/cellulose are being made. Can you say anything about this with the data in hand?*

No, we can not because we collected samples monthly. In the manuscript we described in our methods section that the youngest leaves of a new plant were collected each month. We assume leaves/cellulose are being made at this time (1 month) because Poa flabellata continuously grows.

**Solution:** We added that we assume the leaf cellulose is being made during the past 1 month of growth to the manuscript on Lines 128-129.

*Overall, the discussion is lacking a clear description of how the d13C and d18O would be used to interpret paleoclimate. Is it a temperature signal, a humidity signal, a source of precipitation signal? Or is it all of the above? How will a down core record be interpreted? Is there any way to put some uncertainty into this? How are you going to disentangle the multiple correlations between the isotopes themselves and the relationships with temp and humidity?*

Without experimentation we can not disentangle temperature and humidity using d13C and d18O. We suggest in the manuscript that stomatal conductance is likely driving the changes in d13c and d18O because of relative humidity (Lines 272-273). The precipitation signal can not be separated using these data.

**Solution**: We address these peat core questions in the new section about paleoclimate interpretations (Lines 303-337).

*Figure 1: It would be nice here or elsewhere to have the wind diagrams and the precip source isotopes provided. I don't know what the figure limitations are for this journal, so maybe that's not possible. But, it sure would be nice to have a bit more of the great data collected here summarized in the main article figures.*

Great idea, thank you. We moved both supplemental figures to the main text, as suggested.

*Figure 2: It would be nice if the interpretive strategy figure here was where that data is reported. The peat core is interesting, but not really discussed. It would be nice if it was to put into an interpretive strategy that could be used for downcore paleo Reconstructions.*

We agree and included this in the new section in the Discussion on Lines 303-337and conceptual figure (Fig. 8).

*Figure 3: VSMOW on 3a, but VSMOW and VPDB missing on 3b. For the LMWL reported here, can you report the r or R2, p-value, and n? Figure 4, VSMOW and VPDB needed*

These were all fixed, thank you.

Dear Samuel Bodé,

Thank you for your helpful comments and edits, which have improved this manuscript. We responded to each of your points (blue italic text by providing an explanation of the changes we made in the manuscript.

Kindly,
Dulcinea Groff

**I believe this is an interesting piece of work, as indeed more reliable paleoclimatic proxies for the southern Atlantic are needed, to increase our understanding of past climate patterns. The author also collected a nice dataset. I do agree that Poa flabellate peat has promising potential to be the base of a good proxy, is it has a high accumulation rate in the peat, and is mainly present as the unique plant species. I do however not agree that the real poof of the power of the recorded isotopic signal as paleoclimate proxy has really been given in this manuscript. I have a couple of major concerns on the data treatment and interpretation and a large number of minor remarks.*

*First, the observed correlation of 13C and 18O of the leaf cellulose with RH and T is used as an indication of the power of the proxy for paleo climatic studies. The leaf samples were young leafs growing during the sampled year. The leaves start to grow vertically in the summer and get broader during the winter. The summer samples are thus systematically younger samples than the winter samples. It can not be excluded that the observed difference in 18O and 13C is related to the change in leaf phenology rather than a climatic response. As the entire leaves are collected in winter, the recorded isotopic signal is a combination of the entire growing season.*

We described our collection strategy on Lines 125-129. We collected the youngest leaves of a tussac plant each month to capture the most recent growth (it continuously grows). Because we collected young inner leaf material we do not think the observed pattern is related to change in leaf phenology or systematically younger-older samples.

The time resolution of peat core reconstructions would average several years of accumulated plant matter in a single sample. When measuring d13C and d18O isotopes of cellulose from peat, any seasonal variation would be time-averaged. Alternatively, the measured isotopes could suggest that past environmental conditions were similar to or different from the seasonal relationship we observed in this study. We discuss this point in our new text in the Discussion section on Lines 303-337and in a conceptual figure (Fig. 8).

**Further, it is important to note that this seasonal resolution will not be recorded in the peat record, as only mature leaves will contribute to the litter. A much better way of assessing the potential of the proxy for paleoclimatic reconstructions would of course be to sample a peat core, and correlate 13C and 18O signals of the core to recorded climate data. Therefore, it is needed to better frame the study, and rather use it as a background study on the physiological response of the Tussac grass and incorporation of atmospheric isotopic signal in the cellulose and only put it forward as a very first step toward the development of a paleo climatic proxy.*

We would not assume that only mature leaves contribute to the litter. We think leaves are continuously growing and dying throughout the year.

Because of time averaging (~20-30 years / cm) and limits to historic weather data [dating back to 1874; Lister and Jones 2015], it is not possible to get enough samples to establish a linkage between weather and isotopic values -- we would have only 4-5 peat samples. We agree that paleo data would support this work with a proof of concept, but we wanted to focus on establishing the validity of the proxy through modern calibration, and we think that these actualistic studies are important.

**Further comments: It is not always clear what is tested when statistical tests are performed. An example of this, is when the RH and T of the different locations are compared. It seems to me that the yearly average T and RH are compared using the individual days as replicates (I.e. SD computed on variation between days). This seems wrong to me as the variation in T and RH between individual days has no link with the uncertainty on the average T and RH of that location. To compare the RH and T of the measuring period for these locations only the measurement uncertainty (which is typically very small). Further to be able to say something on the difference in yearly average RH and T between sites in general, several years of observations are needed.*

We are not trying to establish differences in weather conditions between sites, but rather we used multiple sites to test whether there was significant geographic variation in how tussac records T and RH. Our purpose was to show that these analyses can be done on cores from across the study region by establishing a link between T/RH and leaf isotopes.

**Solution:** The new text in Discussion (Lines 303-337) and conceptual figure (Fig. 8) clarify that the space for time substitution used here demonstrates that the processes influencing isotope signals in leaf cellulose observed over a season scales to inter annual change. We demonstrate that the geographic and seasonal patterns give us confidence in the scalability of the relationship between leaf cellulose signals and humidity and temperature.

**The same problem occur when 18O and 2H in precipitation between seasons is compared, the monthly variability is not related to the uncertainty of the mean. On top of this, it is not so meaningful to compare the numerical average when comparing seasons, i.e. the weighted average should be used. Again only the measurement uncertainty is relevant when comparing the seasons. Using the weighed averages the average of the locations could be used to compare the different seasons, however, to me it doesn't seems right to use these different location as replicates.*

It is unclear to us what the reviewer is requesting, so we are not sure how to respond. There may be some confusion about replicates. Our sample sizes are similar between groups. Our use of multiple locations was to determine whether there was any spatial variation in stable isotope values, which would mean any paleoclimate work would require local sampling. That we found no such differences among site responses should be seen as a validation of this approach to the fossil record. We noted in our conceptual figure and new text in the Discussion section (Lines 303-337) that our goal here is not to reconstruct seasonal changes, as peat records will include time-averaging of ~20-30 years/cm.

**as a final note on this seasons start and end the 21th of a month, while samples were taken per month, this should also be acknowledged.) When looking at the correlation of the 13C and 18O in leaf cellulose with RH and T, it is observed that they correlate with both. Beside the issue discussed above it is also important to note that RH and T are also strongly correlated (i.e. Drier (77 -85 %)/hotter (9-11C) summers and wetter (90-98%)/colder (1-5.5C) winters). For which,* *from the data it can not be concluded if the effect is a result of the RH or T or both. It would be interesting to give some insight on what effect might be prevailing here.*

Thanks for taking note of this. **Solution:** We acknowledged this with a new sentence on Lines 155-156: “Because samples were collected at the start of each month, we define summer as the months of DJF and winter as the months JJA.”

For the second part of the comment, we would like to point out that we addressed this point on Lines 269-275.

*Smaller remarks:*
*L75: it sounds quite contradictory to expect low WUE in water-limited environments.*

Thank you for noticing. **Solution**: We edited the sentence to read as, “environments where conservative water use strategies are common functional traits that allow…” on Lines 74-75.

*L80: It is a rather strange thing to say that 18O of source water often correlate with temperature. Better to say (and this is also how it is described in the given reference) that the 18O of precipitation correlates with temperature. (sure in the case of tussocks, source water is directly related to precipitation, but this cannot be claimed in general).*

Agreed, the statement is better revised. Thank you. **Solution:** “The δ18O of source water often correlates with temperature of the environment (Libby et al., 1976).” Lines 79-80.”

*L128: It is not clear what ‘frozen for eight days’ exactly means, what happened after these 8 days? In fact the entire section is not very clear, what is the point of freezing them for 8 days if some where already stored at RT for 6 months? Please rewrite and clarify.*

Agreed, we edited for clarification. **Solution:** “Samples collected between September 2015 to February 2016 were frozen in February 2016 and samples collected in March 2016 to August/September 2016 were frozen in August/September 2016. Samples were frozen for eight days at the Falkland Islands Department of Agriculture to comply with U.S. Department of Agriculture permitting to prevent the spread of pests.” (Lines 125-128).

*L144 and L150: When secondary reference material are used, the accepted value used for it should be given (this do sometimes change over time).*

Thank you. **Solution**: We added this information to Lines 144-150:

“...using USGS-42 (8.6 ‰ accepted value) and IAEA-601 cellulose (31.9‰ accepted value)...”

“...included USGS-40 glutamic acid (-28.3‰ accepted value), USGS-41 glutamic acid (24.4 ‰ accepted value), and internal UWSIF α-cellulose (-24.9 ‰ accepted value).”

*L203: Paragraph is too long, to many irrelevant details are given for which the major lines get lost. I believe this paragraph should be shortened by c.a. 50%*

It is not clear what is being suggested.

*L204: Why is this correlation analysis done on averaged values? It should be done using the individual data points.*

Most correlation statistics assume independence of observations. Using individual data points instead of averaged values would violate this assumption when the goal is to compare seasonal variation across sites each month.

*L13: replace 'investigate' by 'measured', delete 'plants'*

Helpful suggestion, thank you. **Solution:** Rewritten as "Here, we measured the isotopic composition of Poa flabellata and…" on Line 13.

*L14: I believe the author mean: '. . ... explore relationships with seasonal temperature and air humidity variations across 4. . ..'*

Yes, thank you. **Solution:** We changed as described on Line 14.

*L16: Delete 'significantly' (if not you would not report it)*

Good suggestion. We made the change on Line 16..

*L23: 'did not differ significantly' (there is no test to claim that 2 things do not differ, you can only claim that you could not see a significant difference.*

Okay, good suggestion. **Solution:** We changed the sentence to "$\delta^{18}$O values of monthly composite precipitation were similar between seasons or among study locations, yet characteristic of the latitudinal origin of storm tracks and seasonal winds." on Lines 23-24.

*L32: '. . .resulted in an intensification and polward. . ..'*

Okay, we made this change. **Solution**: Changed "correspond" to 'resulted' on Line 32.

*L35: Sentence starting with 'The inconsistency of. . ..' Is not totally clear, reformulate.*

Thanks. We clarified and reformulated by splitting into two sentences. **Solution:** "Meteorological measurements from the Falkland Islands date back to 1874 and are not continuous (Lister and Jones, 2015). This means we lack critical information on the long-term patterns and whether these are novel conditions." on Lines 35-37.

*L47: Should it not be '. . ..generate substantial amounts of peat. . .' ?*

Thanks. We changed this on Line 46 exactly as suggested.

*L57: I beleve it sould be cited as. 'Smith and Prince (1985) established radiocarbon. . .'*

Yes, thank you, we made this change on Line 57.

*L62: '. . ..of any peatland, globally, P. . .. . ."*

Thanks. We made this change on Line 62.

*L67: '. . ..in this semi-arid habitat. . .'*

The references used here do not describe this semi-arid habitat in the Falkland Islands, these studies are about other places in the world that are semi-arid but not the Falkland Islands.

*L69: a) I guess it is 'up to 39 cm' or 'c.a. 39 cm' b) '. . .year) while in winter an increase. . ..'*

We made the recommended change. **Solution**: Changed to "(~ 39 cm per year) while in winter..." on Line 68.

*L70: '. . ..tiller at the base of. . ..'*

Thank you for making this suggestion. **Solution**: Changed to "...production of new tillers at the base of a…" on Line 69.

*L70: Sentence starting with 'The climate signal. . ..' Is not clear, please reformulate.*

Thank you, this sentence was rewritten for clarity. We started a new paragraph here and revised the sentence. **Solution: "**Stable isotopes δ18O, δD and δ13C in the cellulose of plant tissues (roots, shoots, and leaves) can reliably record the climate signal related to environmental growing conditions (Araguás-Araguás et al., 2000)." on Line 70-72.

*L73: 'physiological responses such as changes in stomatal conductance and. . ..'*

Nice suggestion. Will changed accordingly on Lines 73-74.

*L77: '. . .information on. . .'*

Okay, sounds okay either way on Line 77.

*L78: '. . .tissue was formed. . .'*

Okay, change was made on Line 77.

*L79: '. . .humidity and plant physiology (. . .'*

Great suggestion. **Solution**: Changed to "...temperature, humidity, and plant physiology (..." on Line 79.

*L80: '. . .often correlate. . .'*

Good suggestion. . **Solution**: We made the change "...source water often correlates with temperature…" on Line 80.

*L81: '. . .cellulose can also. . .'*

Good suggestion. **Solution:** We made this change. “....cellulose can also be influenced…” on Line 81.

*L82: ‘. . ..plant water pools.’*

Thank you. **Solution**: We made the suggested change “...and plant water pools.” on Line 81.

*L86: rather use ‘‰´*

Good suggestion. We added “(‰)” at first use  on Line 86.

*L97: If mean temperature is given, the time span of this mean should be given, same for*

Good point. **Solution**: Our source (Turner and Pendelbury 2000) references worldclim.org and we added that the time span ranges from 1922-1988 on Line 97.

*L109: Why is the fact that they were first shipped to University of Maine mentioned? I don’t think the reader is interested In the postal rout. . ..*

Okay. **Solution**: We removed this, however it seems relevant to know how the samples were treated.

*L113: It is ‘. . .were purified. . .’ or ‘Water was extracted out of precipitation sample. . ..’*

Good suggestion. **Solution**: We changed to ‘purified’ on Line 113.

*L119: ‘. . .relative to VSMOW.’ No need to mention they are reported in ‰ (this is visible in the results, and can lead to inconsistency).*

Good suggestion. **Solution:** We made this change on Line 118.

*L130: ‘. . .were used, fine roots. . .’*

Okay. We removed the word “and” as suggested on Lines 131-132.

*L133: Just out of personal curiosity, did the grinding of leaf material using a ball mill work? my experience is that this do not work very well with fibrous material.*

The grinding of leaf material with the Retch ball mill worked very well. We used two balls (~ 1 cm diameter ball) in each canister to homogenize the fibrous material.

*L137: a) What is an undetectable amount? Should give a detection limit here, if not it is meaningless. b) it is nitrogen and carbon content, not %nitrogen an %carbon*

Agreed. **Solution**: We edited the sentence like this: “Further indicators of purity include low amounts of nitrogen content (0.13 % to 0.16 % N), and analysis of carbon content (42.1 % to 42.8 % C) in cellulose.” on Lines 136-138.

*L140: ‘varied by < 0.1 ‰ and 0.3 ‰ respectively’*

Good suggestion. **Solution**: We removed the plus/minus signs on Line 140.

*L143: '. . .relative to VSMOW (. . ..'*

Good suggestion. Thank you for helping to tighten this language. **Solution:** The sentence was rewritten as suggested on Line 143.

*L146 and 151: replace ranges by 'c.a. 0.25 mg' and 'c.a. 2 mg'*

Sure. **Solution**: The sentence was edited to reflect the target weigh instead of the range of our weights "c.a. 0.25" and "c.a. 2 mg" on Line 146 and Line 151.

*L148: Delete '; units are expressed. . .. . .. . ..mil)'*

Thanks for the suggestion. **Solution:** We deleted this.

*L150: Reformulate last sentence*

*L153: what is meant with 'analysing the average'? I think the author means that for every site 3 to 4 leafs were used as replicates in every month.*

Our experimental unit was the average of 3-4 plant leaf samples (from new plants each time) at each site each month. We did not expect differences across sites. **Solution**: We edited as suggested "...we averaged three to four plant leaf samples" on Line 154.

*L184: what is the 'n = 344', summer only counts 90 days. . ..*

Thank you for pointing this out. We agree this needed clarification. **Solution**: The sentence was rewritten like this: "...using n = 344 individual trajectories…(n = 332 individual trajectories) were from the…" on Lines 186-188

*L189: Simply say that September 2015 sample was missing for surf bay (we all know that a year has 12 months). Was that sample missing or was it not sampled, meaning that October 2015 is in fact September + October?*

Thank you, this definitely needed clarification. **Solution**: We edited the sentence: "...which was not sampled in September 2015, and October 2015 represents September and October 2015." on Lines 195-196.

*L192: '. . . D values (. . .' , 'monthly composite' is redundant with the first part of the sentence.*

Great catch. Thank you, this improved writing clarity. **Solution**: "D isotopes (y = …)." on Lines 191-192.

*L204: '. . ..leaf 18O and 13C values. . .'*

Thank you. **Solution**: We removed “stable isotopes” so now it reads as“...monthly average leaf δ13C and δ18O values for…” on Lines 208-209.

*L206: add p value after segregation between winter and summer values.*

Thanks. **Solution**: We added our reported p-value from below (Line 216), p < 0.001, to the end of the sentence (Line 208).
“...positive correlation (Pearson’s r = 0.877, p < 0.001, n = 24; Fig. 3B) and segregation between winter and summer values (p < 0.001).” on Line 208.

*L206: How could measurement have a significant correlation? I believe the author meant to write that the d18O value of precipitation did not correlate significantly. Further it would be more logical to write that d18O of leaf and root did not correlate with d18O of precipitation rather than the other way around (statistically it is the same thing, though it is not logical).*

Okay. **Solution**: We changed this to make it more logical: “The δ18Oleaf or δ18Oroot did not correlate with δ18O in precipitation across all…” on Line 208-209.

*L212: ‘28.9 ± 1.3 ‰ídem at*

*L214 etc. . . ..(change everywhere)*

We made these changes. Thanks.

*L213: I do not find it meaning full to add the range, as the distribution was normal, giving average ± SD is enough (and really no reason to add also the range).*

We think both the standard deviation and the range are useful. Reporting the range is valuable for understanding patterns in the data, e.g., boundaries.

*L222: interaction is not an effect, what the author wanted to say is that ‘no significant interaction could be observed’.*

Thanks for making this language clearer. **Solution**: We made this change on Lines 219-220.

*L237: Not clear if this is an own observation (nothing is mentioned about VPD in the result section) or something from literature.*

Thanks for pointing out the need to clarify. **Solution:** We clarified by rewriting to indicate it is information from literature on Line 239.

*L241: ‘. . .ratio of CO2 partial pressure in the leaf and that of the ambient air. . ..’*

Great, thanks. **Solution**: We like this suggestion and edited the sentence like this: “...ratio of CO2 partial pressure in the leaf and that of the ambient air…” Line 243.

*L241: what can be explained, the difference in parial pressure ratio or the effect of it on the 13C?*

Thanks for noting this. We will clarify. **Solution**: We will rewrite like this “Variation in d13C is driven by changes...2000).” on Lines 242-243.

*L257: what are ‘cellulose isotopes’?*

Thanks. **Solution**: We removed “isotopes” on Line 257 and the sentence reads well.

*L317: Quite strange to say ‘at least 12,500 14C years, while on line 64 it says that peatlands initiated between 12,500 and 5,500 14C years. . ..*

Thank you for pointing this out. This sentence could use clarification. **Solution**: We rewrote the first sentence on (Line 63) for consistency (instead of range) with the text “and date back to at least 12,500 14C years,…” found on Line 343.

“…peatlands initiated by 12,500 14C years…” on Line 63

*Figure 3b: Why is not the individual data presented, rather than averages?*

We designed the experiment so that each replicate is one month at each site. In other words, each experimental unit is an average of 3-4 isotope measurements from one site (four total sites).

*Table S1: if the it is given that longitude is south, the negative sign should not be used (sensu stricto a negative south latitiude is a northen latitude). So remove the ‘(S)’ or the ‘-‘ idem for long (W).*

Great suggestion. Thank you for your attention to detail. **Solution**: We removed the “-” from provided latitudes.

*Figure S3: Link did not work, until I found that the ‘-‘ was not for a split for a line break (like my browser interpreted it when clicking on it), but a real hyphen. Probably better to use ‘https://climatereanalyzer.org/’*

Okay, thank you. **Solution**: We updated with the new website name: https://climatereanalyzer.org/

*Figure S5: Add your data to this graph.*

Good suggestion. **Solution**: Based on the suggestion by Reviewer 1, we moved these data to Fig 4. in the main text. The data seem to be best displayed in two panels rather than one plot.

# Modern calibration of *Poa flabellata* (Tussac grass) as a new paleoclimate proxy in the South Atlantic

Dulcinea V. Groff[1,2], David G. Williams[3], Jacquelyn L. Gill[1,2]

[1]Climate Change Institute, University of Maine, Orono, ME 04469, USA
[2]School of Biology and Ecology, University of Maine, Orono, Me 04469, USA
[3]Department of Botany, University of Wyoming, Laramie, WY 82071, USA

*Correspondence to*: Dulcinea V. Groff (dulcineavgroff@gmail.com)

**Abstract.** Terrestrial paleoclimate records are rare in the South Atlantic, limiting opportunities to provide a prehistoric context for current global changes. The tussock grass, *Poa flabellata*, grows abundantly along the coasts of the Falkland Islands and other sub-Antarctic islands. It forms extensive peat records, providing a promising opportunity to reconstruct high-resolution regional climate records. The isotopic composition of leaf and root tissues deposited in these peats has the potential to record variation in precipitation, temperature, and relative humidity over time, but these relationships are unknown for *P. flabellata*. Here, we measured the isotopic composition of *P. flabellata* and precipitation and explore relationships with seasonal temperature and humidity variations across 4 study locations in the Falkland Islands. We reveal that inter-seasonal differences in carbon and oxygen stable isotopes of leaf α-cellulose of living *P. flabellata* correlated with monthly mean temperature and relative humidity. The carbon isotope composition of leaf α-cellulose ($\delta^{13}C_{leaf}$) records the balance of $CO_2$ supply through stomata and the demand by photosynthesis. The positive correlation between $\delta^{13}C_{leaf}$ and temperature and negative correlation between between $\delta^{13}C_{leaf}$ and relative humidity suggest that photosynthetic demand for $CO_2$ relative to stomatal supply is enhanced when conditions are warm and dry. Further, the positive correlation between $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ ($r = 0.88$, $p < 0.001$, $n = 24$) indicates that stomatal closure during warm dry periods explain seasonal variation in $\delta^{13}C_{leaf}$. We observed significant differences between winter and summer seasons for both $\delta^{18}O_{leaf}$ and $\delta^{13}C_{leaf}$, and among study locations for $\delta^{18}O_{leaf}$, but not $\delta^{13}C_{leaf}$. $\delta^{18}O$ values of monthly composite precipitation were similar between seasons and among study locations, yet characteristic of the latitudinal origin of storm tracks and seasonal winds. The weak correlation between $\delta^{18}O$ in monthly composite precipitation and $\delta^{18}O_{leaf}$ further suggests that relative humidity is the main driver of the $\delta^{18}O_{leaf}$. The oxygen isotopes in root α-cellulose did not reflect, or only partially reflected (at one study location), the $\delta^{18}O$ in precipitation. Overall, this study supports the use of peat records formed by *P. flabellata* to fill in a significant gap in our knowledge of the long-term trends in Southern Hemisphere climate dynamics.

## 1 Introduction

The high latitude environments of the South Atlantic are changing rapidly. Over the last century, mean annual temperature in the Falkland Islands (Fig. 1a) has increased by 0.5 ºC (Lister and Jones, 2015). This warming has resulted in an intensification and poleward shift of the southern westerly winds and aridification (Gillett et al., 2008; Thompson and Solomon, 2002; Villalba et al., 2012). These changes are already altering the distribution of marine animals in the Southern Ocean (Weimerskirch et al., 2012), and warming of the western South Atlantic is projected to alter the distribution of island plants as well (Jones et al., 2013; Upson et al., 2016). Meteorological measurements from the Falkland Islands date back to 1874 and are not continuous (Lister and Jones, 2015). This means we lack critical information on the long-term patterns and whether these are novel conditions. Paleoecological archives, such as high-resolution lake sediments and tree rings, can provide useful long-term records documenting and quantifying changes (Dietl et al., 2015; Dietl and Flessa, 2011; Willis et al., 2010), but such records are lacking for the South Atlantic. The absence of trees and deep lakes across many sub-Antarctic islands especially limits high-resolution, independent paleoclimate reconstructions, which are essential for detecting past abrupt climate change. However, many sub-Antarctic islands support widespread communities of peat-forming C3 tussock grasses (*Poa flabellata*), which provide important habitat and shelter for breeding marine animals such as seals and seabirds. Peat records formed by *P. flabellata* present a promising avenue for paleoclimate reconstructions similar to other peatland vegetation types in mid- to high latitudes (Amesbury et al., 2015; Chambers et al., 2012; Pendall et al., 2001).

*P. flabellata* grasslands in the South Atlantic generate substantial amounts of peat (Smith and Clymo, 1984), and have the highest carbon accumulation rates of any peatland globally (Payne et al., 2019). Endemic to the South Atlantic, *P. flabellata* only occurs on Tierra del Fuego, the Falkland Islands, Gough Island, and South Georgia. *P. flabellata* grasslands were once widespread throughout the Falkland Islands, but are now greatly reduced because of land-use change and introduced grazers (Strange et al., 1988; Wilson et al., 1993). The term "tussock" is used to describe the clumping growth form of *P. flabellata,* while the species itself is commonly known as "tussac."

Several factors support the utility of *P. flabellata* peats as a paleoclimate proxy. *P. flabellata* peatlands are formed by a nearly single-species community of *P. flabellata,* which allow very little light or space for other plants to co-occur in the absence of disturbance. Tillers of *P. flabellata* grow on top of a pedestal of decaying roots and leaves that can reach 4 meters high (Fig. 1e) (Smith and Clymo, 1984); mature plants thus likely primarily use water from precipitation, as they are not rooted in the soil directly, or from evaporatively enriched water in the decaying pedestal. Smith and Prince (1985) established radiocarbon ($^{14}$C) dates for a *P. flabellata* pedestal and estimated an age of 250 to 330 years. *P. flabellata* grass forms extensive peat deposits of up to 13.3-m deep, with carbon accumulation rates of 139 g C m$^{-2}$ yr$^{-1}$ (Payne et al., 2019; Smith and Clymo, 1984), far greater than peatlands of similar latitude in the Northern Hemisphere (18.6 g C m$^{-2}$ yr $^{-1}$), the tropics (12.8 g C m$^{-2}$ yr $^{-1}$) or Patagonia (22 g C m$^{-2}$ yr $^{-1}$) (Yu et al., 2010). Subfossil *P. flabellata* leaves are abundant in these peats, and readily can be separated from root subfossils. Having the highest accumulation rate of any peatland, globally, *P. flabellata* peat is ideal for high-resolution climate reconstructions. Basal $^{14}$C radiocarbon dates indicate most *P. flabellata* peatlands initiated by ~ 12,500 $^{14}$C years (Groff, 2018; Payne et al., 2019; Smith and Clymo, 1984).

Grasses exhibiting the tussock growth form often have evergreen leaves and exhibit a profligate/opportunistic water use strategy, due to the high evaporative conditions and pulses of water availability in semi-arid habitats (Moreno-Gutiérrez et al., 2012; Sala et al., 1989; Schwinning and Ehleringer, 2001). The growth phenology of *P. flabellata* is such that it mainly increases in height in summer (~ 39 cm per year) while in winter an increase in basal area occurs with the production of new tillers at the base of the pedestal (Stanworth and Upson, 2013).

Stable isotopes of oxygen ($\delta^{18}O$), hydrogen ($\delta D$), and carbon ($\delta^{13}C$) in the cellulose of plant tissues (roots, shoots, and leaves) can reliably record the climate signal related to environmental growing conditions (Araguás-Araguás et al., 2000). $\delta^{13}C$ and $\delta^{18}O$ record species' water-use strategies in water-limited environments because of physiological responses such as changes in stomatal conductance and assimilation rates (Farquhar and Sharkey, 1982; Moreno-Gutiérrez et al., 2012). Tussock grasses typically occur in water-limited environments where conservative water use strategies are common functional traits that allow tussock grasses to take advantage of pulses of water (Moreno-Gutiérrez et al., 2012). Correlations between $\delta^{18}O$ of plant cellulose and air temperature and humidity provide information on environmental conditions in the season the cellulose tissue was formed. The $\delta^{18}O$ of leaf water is a primary driver of $\delta^{18}O$ in leaf cellulose, and is influenced by the $\delta^{18}O$ value of plant source water, temperature, humidity, and plant physiology (Helliker and Ehleringer, 2002; Roden and Ehleringer, 1999). The $\delta^{18}O$ of source water often correlates with temperature of the environment (Libby et al., 1976). Apart from water source, $\delta^{18}O$ of cellulose can also be influenced by internal exchange among organic molecules and plant water pools (Sternberg et al., 1986). The $\delta^{13}C$ value of leaf biomass in C3 plants records $\delta^{13}C$ of source $CO_2$ and the expression of fractionation effects associated with $CO_2$ diffusion into and through leaf tissue and carboxylation (Farquhar et al., 1982). The net discrimination against $^{13}C$ during photosynthesis is driven by changes in the supply of $CO_2$ through stomatal pores and demand for $CO_2$ by photosynthetic biochemistry (Cernusak et al., 2013; Farquhar et al., 1982; Ferrio and Voltas, 2005). The $\delta^{13}C$ value of roots tends to be 1-3 per mil (‰) higher than that of leaves due to a number of post-photosynthetic biochemical fractionations and C allocation pathways (Cernusak et al., 2009).

Plant species vary in the way they isotopically record precipitation and temperature; therefore, peat comprised of a single species is more desirable over a mixture of species (van Geel and Middeldorp, 1988). To test the potential of *P. flabellata* peatlands as a paleoclimate proxy, we conducted a modern calibration study. We measured $\delta^{18}O$ and $\delta^{13}C$ from living *P. flabellata* leaf tissues (α-cellulose) collected monthly at four sites across the Falkland Islands (51° S, 59° W; Fig. 1b-c). We aim to improve our understanding of Southern Hemisphere climate dynamics with a new paleoclimate proxy that leverages the unique properties of *P. flabellata*.

## 2 Materials and Methods

### 2.1 Study location description

The Falkland Islands (Fig. 1a) are located approximately 500 km east of southern South America, between 51˚0.5' S to 52˚28.0' S and 61˚22.0' W to 57˚40.5' W. The cool-temperate (mean temperature (1922-1988): January 9.4 °C and July 2.2 °C) climate of the Falkland Islands is driven by the cold Antarctic Circumpolar Current, the waters surrounding the Antarctic Peninsula, the Falklands Current, and the Andes of southern Patagonia to the west (Turner and Pendelbury, 2000). The persistent winds of the southwesterly wind belt average 8.5 m $s^{-1}$ (30 km $h^{-1}$), with gale force winds averaging 70 days per year and annual precipitation generally ranges between 400 to 600 mm (Jones et al., 2013; Lister and Jones, 2015). Study sites were selected to reflect 1) climatic diversity, and 2) the availability of volunteers to collect monthly samples for one year. We ultimately selected four sites (Fig. 1b-c): Bleaker Island, Cape Dolphin, Surf Bay, and West Point Island.

## 2.2 Precipitation, temperature and relative humidity

Precipitation was collected at each site using a Palmex monthly composite collection sampler (Palmex d.o.o., Zagbreb, Croatia). The Palmex collector is designed to prevent evaporation and evaporative enrichment of $^{18}$O in precipitation samples without the use of paraffin oil (Gröning et al., 2012), and has been recommended by the Global Network of Isotopes in Precipitation (GNIP). $\delta^{18}$O and $\delta$D stable isotope ratios of water samples were measured at the University of Wyoming Stable Isotope Facility (UWSIF) using a high-temperature conversion elemental analyzer (TCEA) connected to a Thermo Scientific Delta V Plus that is run in continuous flow mode via a ConFlo IV. The technique used injections of 1 µl of water into the TCEA column filled with glassy carbon heated to 1420 °C. Precipitation samples were purified using cryogenic vacuum distillation (Ehleringer and Osmond, 1989) prior to TCEA analysis to remove aeolian debris, including marine salts. Internal QA/QC working standards calibrated against IAEA international standards Vienna Standard Mean Ocean Water (VSMOW) and Standard Light Antarctic Precipitation (SLAP) and spanning the range of measured values in our study were analyzed with each batch of samples with analytical precision typically better than 0.3 and 2.5 ‰ for $\delta^{18}$O and $\delta$D, respectively. Isotope values are reported with respect to VSMOW. Explanations of methods for daily average temperature and relative humidity measurements, as well as seasonal wind speed, wind direction, and back trajectory models to determine origins of air masses are found in the Supplemental Text 1.

## 2.3 *Poa flabellata* field collection and cellulose extraction, and isotope analyses

*Poa flabellata* plants were collected at the start of each month at each site from October 2015 through September 2016, from relatively uniform habitats that were undisturbed by grazing or tilling. Up to six *P. flabellata* plant tillers (leaves, stem, and roots) were collected near each of the four stations each month. Whole plants were stored in paper envelopes stored in a cool, dark, dry location until frozen. Samples collected between September 2015 to February 2016 were frozen in February 2016 and samples collected in March 2016 to August/September 2016 were frozen in August/September 2016. Samples were frozen for eight days at the Falkland Islands Department of Agriculture to comply with U.S. Department of Agriculture permitting to prevent the spread of pests. For leaf material, the inner developing (youngest) leaves were collected and assumed to represent the past month of growth. There was no indication that leaves were morphologically different between summer and winter. Only coarse roots were used, fine roots were excluded. Variation in environmental conditions during the growth of a leaf blade can lead to isotopic variations along the gradient of a single leaf as has been shown with $\delta^{18}$O of cellulose (Helliker and Ehleringer, 2000, 2002); therefore, whole-leaf plant samples were homogenized by drying at 50 °C and pulverizing using a Retsch ball mill at the University of Maine. For each sample, we used 20 mg of pulverized and homogenized leaf or root material for extraction and purification of α-cellulose, following an adapted procedure of (Brendel et al., 2000). Samples were vortexed throughout extraction and purification for homogenization and were visually inspected for purity. Further indicators of purity include low amounts of nitrogen content (0.13 % to 0.16 % N), and analysis of carbon content

(42.1 % to 42.8 % C) in cellulose. As an internal quality control, one leaf sample was selected for extraction and purification of α-cellulose throughout the sample processing in batches of 10 to 12 samples. The $\delta^{13}C$ and $\delta^{18}O$ of leaf cellulose for the internal quality control samples varied by < 0.1 ‰ and 0.3 ‰, respectively.

$\delta^{18}O$ and $\delta^{13}C$ stable isotope ratios of α-cellulose samples were measured at the University of Wyoming Stable Isotope Facility (UWSIF). Oxygen was analyzed using a TCEA coupled to a Thermo Delta V IRMS; $\delta^{18}O$ values are expressed relative to VSMOW (Craig, 1961; Gonfiantini, 1978). Values were normalized to the VSMOW scale using USGS-42 (8.6 ‰ accepted value) and IAEA-601 cellulose (31.9 ‰ accepted value) quality control standard reference materials for oxygen isotopic composition. Analytical precision was ± 0.3 ‰ for $\delta^{18}O$ based on repeated analysis of internal standards, and samples loaded into silver capsules had weights ca. 0.25 mg. Carbon isotope composition of the cellulose samples were determined using a Costech 4010 Elemental Analyzer coupled to a Thermo Delta Plus XP-IRMS. Analytical precision was ± 0.1 ‰ for $\delta^{13}C$ based on repeated internal standards. Quality control standard reference material for carbon isotopic composition included USGS-40 glutamic acid (-28.3‰ accepted value), USGS-41 glutamic acid (24.4 ‰ accepted value), and internal UWSIF α-cellulose (-24.9 ‰ accepted value). Carbon samples weighed ca. 2 mg and were loaded into tin capsules.

### 2.4 Statistical analysis

For both $\delta^{18}O$ and $\delta^{13}C$, we analysed the average of three to four plant leaf samples per month for summer (DJF) and winter (JJA) season at each of the four sites, and the average of up to eight plant root samples (Supplementary Data 1 and 2). Because samples were collected at the start of each month, we define summer as the months of DJF and winter as the months JJA. We used Pearson's correlation coefficient, r, to detect associations between $\delta^{18}O$ of cellulose and precipitation samples to test whether $\delta^{18}O$ samples reflects the isotopic value of precipitation. Using Pearson's correlation coefficient, we tested for a relationship between $\delta^{13}C$ and $\delta^{18}O$ values of cellulose, temperature, and relative humidity. We tested for a significant difference between summer and winter $\delta^{18}O$ and $\delta D$ in precipitation using a t-test ($n$ = 24). A separate one-way analysis of variance (ANOVA) to test for significant differences among sites included $\delta^{18}O$ and $\delta D$ in precipitation samples from the entire year ($n$ = 47).

A two-way ANOVA compared the main effects of season (summer vs. winter) and the four study locations on the $\delta^{18}O$ and $\delta^{13}C$ stable isotopes of α-cellulose of *P. flabellata* leaves and roots grown in the summer versus winter, followed by a post hoc test (Tukey's multiple comparison of means). P-values < 0.05 are considered significant. Descriptive and multivariate analyses were conducted with SigmaPlot 12.5.

## 3 Results

### 3.1 Environmental measurements

Across all sites, summer daily average temperatures ranged from 3.5 °C to 15.6 °C (mean = 10.0 °C) and relative humidity ranged from 64.2 % to 98.1 % (mean = 81.1 %). Winter daily average temperatures ranged from -1.8 °C to

7.6 °C (mean = 3.7 °C), and relative humidity ranged from 73.6 % to 100 % (mean 94.3 %). Seasonal temperature (°C) and relative humidity (%) minimum and maximum ranges for individual study locations are found in Table S1. Between study locations, the daily average temperatures over the year ($F_{(3, 44)} = 0.316$, $p = 0.813$, Fig. 2a) and relative humidity were not significantly different ($F_{(3, 44)} = 0.674$, $p = 0.573$, Fig. 2b).

### 3.2 Wind

The wind rose (Fig. 1d) shows that winter winds at Bleaker Island primarily blew from the west and northwest. In winter, two spokes in the west and NNW direction comprise >30% of the total recorded 15-minute wind directions. In summer, three spokes in the west, WSW, and SW directions comprise >45% of all 15-minute wind directions. The wind rarely blew from the east, SE, or north. Examining winds from the west in winter, >10% of wind speeds recorded were between 5 and 10 m $s^{-1}$ (18 and 36 km $h^{-1}$), and the frequency of strongest winds came from the NNE. In summer, >20% winds from the SW were between 5 and 10 m $s^{-1}$, and there was a higher frequency of 10 to 15 m $s^{-1}$ (36 to 54 km $h^{-1}$) wind speeds than in winter. Seasonal wind variation deviated from the long-term average (1979-2015). Reanalysis data (ERA Interim; Fig. S2) indicated that the wind speeds during summer (DJF 2015 to 2016) were stronger over the Falkland Islands (5 to 6 m $s^{-1}$) (18 to 21 km $h^{-1}$) and weaker during winter (JJA 2016).

### 3.3 Seasonal HYSPLIT air mass trajectory analyses

The daily back trajectory HYSPLIT analysis indicated that during the summer, 89% of the air masses originated ($n$ = 344 individual trajectories) west of the Falkland Islands. Approximately 11% of summer air masses originated south of the Falkland Islands near the Antarctic Peninsula. In winter, 79% of the air mass back trajectories ($n$ = 332 individual trajectories) were from the west, NW, and SW, while 21% of air masses had backward trajectories south of the Falkland Islands near the Antarctic Peninsula (Fig. 3).

### 3.4 Monthly composite precipitation, $\delta^{18}$O and $\delta$D

Each study location had $n$ = 12 samples over the year, except for Surf Bay ($n$ = 11), which was not sampled in September 2015, and October 2015 represents September and October 2015. Monthly composite $\delta^{18}$O and $\delta$D isotopes in precipitation throughout the year ranged from -12.3 ‰ to -4.8 ‰, and from -86 ‰ to -23 ‰, respectively. Monthly composite precipitation at each location was used to construct a local meteoric water line using $\delta^{18}$O and $\delta$D isotopes ($y = 7.571x + 5.527$; $n = 47$; Fig. 4a). The range for winter $\delta^{18}$O and $\delta$D was from -8.6 ‰ to -6.6 ‰ and -61 ‰ to -40 ‰, respectively. Summer values of $\delta^{18}$O and $\delta$D in precipitation ranged from -12.3 ‰ to -5.3 ‰ and -86 ‰ to -38 ‰, respectively, and fit within the range of historical isotopes in precipitation from the Falkland Islands (GNIP; Fig. 4b). Summer and winter $\delta^{18}$O and $\delta$D isotopes in precipitation ($n$ = 24) passed tests for normality (Shapiro-Wilk, $p = 0.297$ and $p = 0.614$, respectively) and failed tests for equal variance (Fisher's F test, $p < 0.05$). A Mann-Whitney Rank Sum test indicated that the $\delta^{18}$O isotopes in precipitation were not different for summer (median = -8.3 ‰) and winter (median = -7.4 ‰, U = 39, $p = 0.061$). For $\delta$D, the summer had a significantly lower median value (median = -64.3 ‰) than winter (median = -46.5 ‰, U = 22, $p = 0.004$). A one-way ANOVA found no significant difference among sites in $\delta^{18}O$ ($F_{(3,43)} = 0.323$, $p = 0.809$) or $\delta D$ isotopes ($F_{(3,43)} = 0.361$, $p = 0.785$) in precipitation when samples from all months and sites were included ($n = 47$).

### 3.5 $\delta^{13}C$ and $\delta^{18}O$ of α-cellulose – temperature, humidity, precipitation

Across all sites, measurements of monthly average leaf $\delta^{18}O$ and $\delta^{13}C$ values for α-cellulose extracted from leaf tissues (hereafter $\delta^{18}O_{leaf}$ and $\delta^{13}C_{leaf}$) had a strong positive correlation (Pearson's $r = 0.877$, $p < 0.001$, $n = 24$; Fig. 5) and segregation between winter and summer values ($p < 0.001$). The $\delta^{18}O_{leaf}$ or $\delta^{18}O_{root}$ did not correlate with $\delta^{18}O$ in precipitation across all sites (Table 1).

$\delta^{18}O_{leaf}$ and $\delta^{13}C_{leaf}$ values passed tests for normality (Shapiro-Wilk, $p = 0.173$ and $p = 0.385$, respectively) and equal variance (Fisher's F test, $p = 0.865$ and $p = 0.196$, respectively). Thus, a two-way analysis of variance was conducted to detect the influence of independent variables (season and study location) on both $\delta^{18}O_{leaf}$ and $\delta^{13}C_{leaf}$. Season included two levels (summer and winter) and study location consisted of four levels (Bleaker Island, Cape Dolphin, Surf Bay, and West Point Island). Analysis of combined winter and summer $\delta^{18}O_{leaf}$ had a mean of 28.9 ± 1.3 ‰ (SD), and ranged from 26.3 ‰ to 31.8 ‰ (range of 5.4 ‰; Table S2). The effect of season was significant ($F_{(1, 16)} = 183.2$, $p < 0.001$) with a 2.6 ‰ difference between summer (mean = 30.1 ± 0.8 ‰ (SD)) and winter (mean = 27.5 ± 0.6 ‰ (SD); Fig. 6; Table S2). The effect of study location was also significant ($F_{(3, 16)} = 4.8$, $p = 0.014$) in $\delta^{18}O_{leaf}$ among study locations. Pairwise multiple comparison (Tukey's post hoc test) of study locations indicated that Surf Bay is significantly more depleted in $^{18}O_{leaf}$ than Cape Dolphin ($p = 0.016$) and Bleaker Island ($p = 0.029$; Fig. 6). No significant interaction was observed ($p = 0.552$). The mean of combined winter and summer $\delta^{13}C_{leaf}$ value was -25.4 ± 1.31 ‰ (SD), ranging from -30.4 ‰ to -21.9 ‰ (range = 8.4 ‰; Table S2). For $\delta^{13}C_{leaf}$, there was a significant difference between seasonal values (F ratio of $F_{(1, 16)} = 40.8$, $p < 0.001$) in summer (mean = -24.2 ± 1.05 ‰ (SD)) and winter (mean = -26.8 ± 1.3 ‰ (SD), Fig. 6; Table S2). Study location ($p = 0.861$; Figs. 6b and 6d) and the interaction effect ($p = 0.638$) were not significant. The mean $\delta^{13}C$ in root α-cellulose (hereafter $\delta^{13}C_{root}$; $n = 14$) for summer was -25.3 ± 1.27 ‰ (SD), and -26.6 ± 1.38 ‰ (SD) in winter (Table S2). After $\delta^{13}C_{root}$ data passed tests for normality (Shapiro-Wilk test; $p = 0.085$), but not equal variance ($p < 0.05$), the two-way ANOVA indicated that for $\delta^{13}C_{root}$, the effects for season ($p = 0.201$) and study location ($p = 0.521$) were not statistically significant. The interaction effect was not significant ($p = 0.886$).

The mean $\delta^{18}O$ in root α-cellulose (hereafter $\delta^{18}O_{root}$; $n = 14$) for summer was 28.8 ± 1.04 ‰ (SD), and 28.3 ± 0.5 ‰ (SD) for winter (Table S2). The $\delta^{18}O_{root}$ data passed tests for normality (Shapiro-Wilk test; $p = 0.483$) and equal variance (Fisher's F test; $p = 0.897$); the two-way ANOVA indicated that for $\delta^{18}O_{root}$, the location effect was statistically significant, while season was not. The difference in mean values among seasons ($F_{(1, 8)} = 5.4$, $p = 0.049$) and study location ($F_{(2, 8)} = 8.7$, $p = 0.010$) were statistically significant. Pairwise multiple comparison (Tukey's post hoc test) of study locations indicated that Cape Dolphin was significantly greater than Bleaker Island ($p = 0.012$) and West Point Island ($p = 0.049$). The interaction effect was not significant ($p = 0.397$).

## 4 Discussion

Significant inter-seasonal differences in $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ indicate that *P. flabellata* tissues record high-resolution patterns of environmental change, supporting the use of *P. flabellata* peat records as a paleoenvironmental proxy. Ferrio and Voltas (2005) established a positive correlation between $\delta^{13}C_{leaf}$ and vapor pressure deficit suggesting stomatal conductance is sensitive to atmospheric moisture conditions. The observed positive correlation between $\delta^{13}C_{leaf}$ and temperature suggests higher temperatures led to an increased assimilation rate and reduced discrimination against $\delta^{13}C$ as shown in other vascular plant studies (Ferrio and Voltas, 2005; Ménot and Burns, 2001). Variation in $\delta^{13}C_{leaf}$ is driven by changes in the ratio of $CO_2$ partial pressure in the leaf and that of the ambient air, and can be explained by a greater influence of either stomatal conductance or increased photosynthetic capacity (Scheidegger et al., 2000). As plant stomata close in response to low humidity and/or high evaporative conditions like high wind speeds in the Falkland Islands, the internal partial pressure of $CO_2$ decreases and the $\delta^{13}C_{leaf}$ increases (Farquhar et al., 1982, p.198).

The $\delta^{18}O_{leaf}$ is influenced by soil water, leaf water enrichment of $^{18}O$ from transpiration, and biochemical fractionations. Leaf water enrichment of $^{18}O$ due to transpiration, which is reflected in $\delta^{18}O_{leaf}$ (Deniro and Epstein, 1979; Roden and Ehleringer, 1999; Sternberg et al., 1986, p.198; Yakir, 1992), depends on relative humidity (Helliker and Ehleringer, 2002). However, the relationship between relative humidity and $\delta^{18}O_{leaf}$ deteriorated at relative humidity > 90 % in one C3 species (Helliker and Ehleringer, 2002). Diffusion limitation by stomatal resistance is primarily driven by relative humidity (White et al., 1994). The $\delta^{18}O_{leaf}$ and relative humidity (> 60 %) were negatively correlated, which is consistent with other studies showing that $\delta^{18}O_{leaf}$ increases as relative humidity decreases (Barbraud et al., 2012; Helliker and Ehleringer, 2002). At high relative humidity the leaf will more strongly record variation in the isotopic composition of atmospheric vapor, however we have no direct measurements of $\delta^{18}O$ in water vapor.

Previous work on leaf water and cellulose in grasses demonstrated that the atmospheric-leaf vapor conditions are a strong predictor of $\delta^{18}O_{leaf}$ (Helliker and Ehleringer, 2002; Lehmann et al., 2018). The $\delta^{18}O$ of the leaf water is captured in the cellulose isotopes and can reflect the effect of changing environmental conditions during the growth of the leaf (Helliker and Ehleringer, 2002; Lehmann et al., 2017). The $\delta^{18}O_{leaf}$ can also depend on physiological effects, the type of plant anatomical feature used, and stage of development (Lehmann et al., 2017; Liu et al., 2017). Because cellulose records environmental variation along a gradient during leaf growth, we collected and homogenized whole leaves to avoid the complications of $\delta^{18}O$ enrichment (Helliker and Ehleringer, 2002). Our work supports the finding that atmospheric-leaf vapor conditions are reflected in $\delta^{18}O_{leaf}$, and expands the use of such paleoclimate proxies to peat-forming tussock grasses, which opens up new possibilities for reconstructing paleoclimates across the South Atlantic and beyond. At the higher relative humidity range, the $\delta^{18}O_{leaf}$ is more of a reflection of source water, while the $\delta^{18}O_{leaf}$ at low humidity differed greatly from source waters because of evaporative enrichment of $^{18}O$.

The positive correlation between $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ (Pearson's r = 0.88, $p < 0.001$, $n = 24$; Table 1; Fig. 5 suggests that stomatal conductance is the driving force acting on these two proxies, which is a likely scenario when water is not limiting (Saurer et al., 1997; Scheidegger et al., 2000). According to the Scheidegger et al.(2000) model, the decline in stomatal conductance was much more strongly expressed than photosynthetic capacity (maximum net photosynthesis). When air humidity increases, stomatal conductance is assumed to increase. In our study, stomatal conductance is likely driving both $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ due to relative humidity (Fig. 7). This pattern fits well with the Barbour and Farquhar (2000) model. Measurements of relative humidity allowed us to determine that stomatal conductance was more influential as a possible cause of change in partial pressure of $CO_2$ within the leaf.

In the Falkland Islands, precipitation amount is not highly seasonal, but tends to vary the most in the summer (Fig. S3; data from Jones et al., 2013). Over the year of our study, summer $\delta^{18}O$ in precipitation varied more than winter $\delta^{18}O$ and tended to be more depleted. This is supported by the wind rose from Bleaker Island, which indicated prevailing winds from the SW (Fig. 1d). In winter, precipitation tended to be more enriched in $^{18}O$, and most prevailing winds came from the west and NW where $^{18}O$ in equatorward precipitation would be more enriched than $^{18}O$ in high latitude meteoric sources (Fig. 4b). The significant negative correlation between $\delta^{18}O$ in precipitation and $\delta^{18}O_{root}$ at Cape Dolphin (Pearson's r = 0.868, p = 0.025, $n = 6$; Table 1) is consistent with the latitudinal origin of storm tracks (Fig. 3) and seasonal wind data (Fig. 1d). The pattern found at Bleaker Island is less clear, and warrants further investigation.

The observed lack of correlations between $\delta^{18}O_{leaf}$ and $\delta^{18}O$ values of precipitation (Table 1) demonstrate the overriding influence of humidity on patterns of leaf water $\delta^{18}O$. An alternative explanation for a lack of correlation is that our precipitation sampling density was not sufficient to establish a relationship between $\delta^{18}O_{leaf}$ and precipitation $\delta^{18}O$. Although *P. flabellata* produces new leaves throughout the year, the growth rate of leaves may not be in sync with shorter precipitation sampling intervals (less than monthly composite precipitation). Examination of leaf waters post-precipitation events would improve our understanding of $\delta^{18}O_{leaf}$.

The observed relationship between $\delta^{18}O$ in precipitation and $\delta^{18}O_{root}$ appeared to be less clear in part due to low sample number from only three study locations (Bleaker Island, Cape Dolphin, and West Point Island). At Bleaker Island, there was no correlation between $\delta^{18}O$ in precipitation and $\delta^{18}O_{root}$, while Cape Dolphin had a strong negative correlation (Table 1). At Cape Dolphin, greatest enrichment of $^{18}O_{root}$ occurred in summer when $\delta^{18}O$ precipitation was relatively low. In contrast, at Cape Dolphin $\delta^{18}O_{root}$ was lowest during the winter months when $\delta^{18}O$ of precipitation was high. Despite the strong relationship between $\delta^{18}O$ precipitation and $\delta^{18}O_{root}$ at Cape Dolphin, we propose that *P. flabellata* peatlands may not be ombrotrophic, and may potentially source water from fog, sea-spray, groundwater, or a mix. However, the relationship found at Cape Dolphin warrants further analysis of $\delta^{18}O_{root}$ and source waters. We also consider that the $\delta^{18}O_{root}$ records a signal of leaf humidity, and is influenced by source water and humidity effects on leaves; sugars produced in leaves are transported to roots to form cellulose, and about half of the oxygen atoms in root cellulose originate from the leaf water signal. Roots of *P. flabellata* may have represented greater temporal integration of $\delta^{18}$O from precipitation into cellulose due to difficulty in distinguishing new growth in roots, like *Empodisma* in New Zealand (Amesbury et al., 2015).

We did not find a relationship between $\delta^{18}$O in precipitation and the $\delta^{18}O_{leaf}$ in our one-year study. It may be that because the $\delta^{18}$O in leaf water is controlled by source water and humidity, any changes in humidity confound a direct relationship between source water $\delta^{18}$O values and $\delta^{18}$O of leaf water and $\delta^{18}O_{leaf}$. Still, the $\delta^{18}$O in plant water pools and $\delta^{18}O_{leaf}$ are primarily influenced by $\delta^{18}$O in precipitation. It may also be possible that the variation in source area did not affect the $\delta^{18}$O enough for us to detect a significant impact on the $\delta^{18}O_{leaf}$. Based on this work, the source area could influence $\delta^{18}O_{leaf}$ in peat records if source area changes were greater than what we observed. Without experimentation we can not disentangle temperature and humidity using $\delta^{13}$C and $\delta^{18}$O; further work is needed to understand the relationship between $\delta^{18}O_{root}$, $\delta^{18}$O of precipitation, and that of root and leaf waters. Identifying sources of potential water would also add value, especially considering anecdotes of local differences in fog in the Falkland Islands, which may be an unappreciated source of water for *P. flabellata*. Across the geographic range in the South Atlantic, *P. flabellata* may record a larger latitudinal gradient of isotopes in precipitation, as well as temperature and humidity, than recorded in the Falkland Islands.

Despite this limitation, establishing the seasonal patterns recorded by *P. flabellata* cellulose in the Falkland Islands does enable us to test paleoclimate hypotheses regarding the climate dynamics in the South Atlantic (e.g. Turney et al., 2016) and Southern Hemisphere westerly wind behavior within the regions where *P. flabellata* occurs. As with any paleoenvironmental reconstruction, inferences are constrained by the temporal grain and resolution (Jackson, 2012). While *P. flabellata* is sensitive to inter-seasonal differences and forms highly productive peat records, we stress that paleoclimate reconstructions from *P. flabellata* peats will represent an integrated signal of broader climate trends, and not annual-scale or seasonal records. Our calibration study is based on a modern snapshot of environmental conditions influencing $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ values in *P. flabellata*, so investigators conducting downcore peat reconstructions using $\delta^{18}O_{leaf}$ must consider that changes in $\delta^{18}O_{leaf}$ are modified by precipitation source and changes in humidity through effects on stomatal conductance. We recommend measuring $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ of subsamples with comparable time-averaging (leaf, root, bulk subfossils; Fig. 8) by incorporating a large sampling of multiple leaf fragments ($n \geq 10$) from each horizon (e.g. 1 cm intervals), and to interpret this signal to integrate multiple years (as determined by the sediment accumulation rate; Fig. 8). Time-averaging within a given 1 cm horizon of even highly productive peat records with sedimentation rates ~ 20 to 30 yr cm$^{-1}$ would be greater than the age of an individual tussac leaf.

Peat-based reconstructions may be limited to identifying periods of warm/dry or cold/wet conditions that are similar to (or more extreme than) the observed seasonal variations we report here. Thus, we believe that $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ time-averaged values can reliably be used to identify the timing of transitions between warmer and drier conditions to colder and wetter conditions that correlate with major hemispheric drivers in climate. Resolving temperature and moisture signals independently would likely require growth chamber or warming experiments, which was beyond the scope of this study, but which could help develop this proxy further. For now, this proxy remains a qualitative indicator, though it has potential to become a quantitative reconstruction if evaluated via experimentation or in tandem with other plant or microbial biomarkers.

## 5 Conclusion

The scarcity of terrestrial paleoclimate records in the South Atlantic has limited our understanding of past and future climate change and its impacts on ecosystems and people. We found that $\delta^{13}C$ and $\delta^{18}O$ stable isotope values in *P. flabellata* tissues are correlated with seasonal differences in temperature and moisture, providing a promising new avenue for paleoclimate reconstructions in the South Atlantic. *P. flabellata* peats have high accumulation rates, contain abundant leaves, and date back to at least 12,500 $^{14}C$ years, with the potential to provide decadal-scale records of temperature, precipitation, and moisture source. Future work is needed to determine whether $\delta^{18}O$ and $\delta^{13}C$ of cellulose from *P. flabellata* macrofossils complement other regional proxies for changes in atmospheric temperature and relative humidity during the Holocene. Troublingly, these coastal peatlands are currently threatened by sea level rise and over-grazing, and their reductions means we are losing vital information about past environments in a time when paleoclimate records are needed to provide context for modern climate change in the South Atlantic.

## 6 Data availability

Datasets for monthly stable isotopes in precipitation, average temperature, and average relative humidity have been submitted to the Global Network of Isotopes in Precipitation (https://nucleus.iaea.org/wiser) and will be publicly available upon acceptance for publication. Datasets for leaf and root stable isotopes of cellulose can be found at http://dx.doi.org/10.5281/zenodo.3104573, hosted at Zenodo upon acceptance for publication.

## 7 Author contribution

DG, DW, and JG designed the experiments and DG carried them out. DG performed laboratory analyses. DG prepared the manuscript with contributions from all co-authors.

## 8 Competing interests

The authors declare that they have no conflict of interest.

## 9 Acknowledgements

We gratefully thank the citizen scientists who collected samples: Ben Bernsten, Nikki and Mike Summers, Mike and Phyl Rendell, Robert and Elaine Short, Kicki Ericson, Thies Matzen. Paul Mayewski at the University of Maine Climate Change Institute provided precipitation collectors. Logistical, field, and laboratory support was provided by

Paul Brickle and Megan Tierney at the South Atlantic Environmental Research Institute, Craig Cook at the University of Wyoming Stable Isotope Facility, Kayla Greenawalt, and Jiemin Guo. Funding: This research was supported by the US National Science Foundation [grant numbers DGE-1144423 and EF-1137336].

## 10 References

Amesbury, M. J., Charman, D. J., Newnham, R. M., Loader, N. J., Goodrich, J. P., Royles, J., Campbell, D. I., Roland, T. P. and Gallego-Sala, A.: Carbon stable isotopes as a palaeoclimate proxy in vascular plant dominated peatlands, Geochim. Cosmochim. Acta, 164, 161–174, doi:10.1016/j.gca.2015.05.011, 2015.

Araguás-Araguás, L., Froehlich, K. and Rozanski, K.: Deuterium and oxygen-18 isotope composition of precipitation and atmospheric moisture, Hydrol. Process., 14(8), 1341–1355, doi:10.1002/1099-1085(20000615)14:8<1341::AID-HYP983>3.0.CO;2-Z, 2000.

Barbraud, C., Rolland, V., Jenouvrier, S., Nevoux, M., Delord, K. and Weimerskirch, H.: Effects of climate change and fisheries bycatch on Southern Ocean seabirds: a review, Mar. Ecol. Prog. Ser., 454, 285–307, doi:10.3354/meps09616, 2012.

Brendel, O., Iannetta, P. P. M. and Stewart, D.: A rapid and simple method to isolate pure alpha-cellulose, Phytochem. Anal., 11(1), 7–10, doi:10.1002/(SICI)1099-1565(200001/02)11:1<7::AID-PCA488>3.0.CO;2-U, 2000.

Cernusak, L. A., Tcherkez, G., Keitel, C., Cornwell, W. K., Santiago, L. S., Knohl, A., Barbour, M. M., Williams, D. G., Reich, P. B., Ellsworth, D. S., Dawson, T. E., Griffiths, H. G., Farquhar, G. D. and Wright, I. J.: Why are non-photosynthetic tissues generally 13C enriched compared with leaves in C3 plants? Review and synthesis of current hypotheses, Funct. Plant Biol., 36(3), 199, doi:10.1071/FP08216, 2009.

Cernusak, L. A., Ubierna, N., Winter, K., Holtum, J. A. M., Marshall, J. D. and Farquhar, G. D.: Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants, New Phytol., 200(4), 950–965, doi:10.1111/nph.12423, 2013.

Chambers, F. M., Booth, R. K., De Vleeschouwer, F., Lamentowicz, M., Le Roux, G., Mauquoy, D., Nichols, J. E. and van Geel, B.: Development and refinement of proxy-climate indicators from peats, Quat. Int., 268, 21–33, doi:10.1016/j.quaint.2011.04.039, 2012.

Craig, H.: Standard for Reporting Concentrations of Deuterium and Oxygen-18 in Natural Waters, Science, 133(3467), 1833–1834, doi:10.1126/science.133.3467.1833, 1961.

Deniro, M. J. and Epstein, S.: Relationship Between the Oxygen Isotope Ratios of Terrestrial Plant Cellulose, Carbon Dioxide, and Water, Science, 204(4388), 51–53, doi:10.1126/science.204.4388.51, 1979.

Dietl, G. P. and Flessa, K. W.: Conservation paleobiology: putting the dead to work, Trends Ecol. Evol., 26(1), 30–37, doi:10.1016/j.tree.2010.09.010, 2011.

Dietl, G. P., Kidwell, S. M., Brenner, M., Burney, D. A., Flessa, K. W., Jackson, S. T. and Koch, P. L.: Conservation Paleobiology: Leveraging Knowledge of the Past to Inform Conservation and Restoration, Annu. Rev. Earth Planet. Sci., 43(1), 79–103, doi:10.1146/annurev-earth-040610-133349, 2015.

Ehleringer, J. R. and Osmond, C. B.: Stable isotopes, in Plant Physiological Ecology: Field methods and instrumentation, edited by R. W. Pearcy, J. R. Ehleringer, H. A. Mooney, and P. W. Rundel, pp. 281–300, Springer Netherlands, Dordrecht., 1989.

Farquhar, G., O'Leary, M. and Berry, J.: On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves, Funct. Plant Biol., 9(2), 121, doi:10.1071/PP9820121, 1982.

Farquhar, G. D. and Sharkey, T. D.: Stomatal Conductance and Photosynthesis, Annu. Rev. Plant Physiol., 33(1), 317–345, doi:10.1146/annurev.pp.33.060182.001533, 1982.

Ferrio, J. P. and Voltas, J.: Carbon and oxygen isotope ratios in wood constituents of Pinus halepensis as indicators of precipitation, temperature and vapour pressure deficit, Tellus B, 57(2), 164–173, doi:10.1111/j.1600-0889.2005.00137.x, 2005.

van Geel, B. and Middeldorp, A. A.: Vegetational history of Carbury Bog (Co. Kildare, Ireland) during the last 850 years and a test of the temperature indicator value of 2H/1H measurements of peat samples in relation to historical sources and meteorological data, New Phytol., 109(3), 377–392, doi:10.1111/j.1469-8137.1988.tb04208.x, 1988.

Gillett, N. P., Stone, D. A., Stott, P. A., Nozawa, T., Karpechko, A. Yu., Hegerl, G. C., Wehner, M. F. and Jones, P. D.: Attribution of polar warming to human influence, Nat. Geosci., 1(11), 750–754, doi:10.1038/ngeo338, 2008.

Gonfiantini, R.: Standards for stable isotope measurements in natural compounds, Nature, 271(5645), 534–536, doi:10.1038/271534a0, 1978.

Groff, D. V.: Modern and Paleoecological Perspectives on a Terrestrial-Marine Linkage in the Falkland Islands, PhD Thesis Univ. Maine, 196, 2018.

Gröning, M., Lutz, H. O., Roller-Lutz, Z., Kralik, M., Gourcy, L. and Pöltenstein, L.: A simple rain collector preventing water re-evaporation dedicated for δ18O and δ2H analysis of cumulative precipitation samples, J. Hydrol., 448–449, 195–200, doi:10.1016/j.jhydrol.2012.04.041, 2012.

Helliker, B. R. and Ehleringer, J. R.: Establishing a grassland signature in veins: 18O in the leaf water of C3 and C4 grasses, Proc. Natl. Acad. Sci., 97(14), 7894–7898, doi:10.1073/pnas.97.14.7894, 2000.

Helliker, B. R. and Ehleringer, J. R.: Grass blades as tree rings: environmentally induced changes in the oxygen isotope ratio of cellulose along the length of grass blades, New Phytol., 155(3), 417–424, doi:10.1046/j.1469-8137.2002.00480.x, 2002.

Jackson, S. T.: Representation of flora and vegetation in Quaternary fossil assemblages: known and unknown knowns and unknowns, Quat. Sci. Rev., 49, 1–15, doi:10.1016/j.quascirev.2012.05.020, 2012.

Jones, P. D., Harpham, C. and Lister, D. H.: Construction of high spatial resolution climate scenarios for the for the Falkland Islands and southern Patagonia, U. K. Falkl. Isl. Trust, 40, 2013.

Lehmann, M. M., Gamarra, B., Kahmen, A., Siegwolf, R. T. W. and Saurer, M.: Oxygen isotope fractionations across individual leaf carbohydrates in grass and tree species, Plant Cell Environ., 40(8), 1658–1670, doi:10.1111/pce.12974, 2017.

Lehmann, M. M., Goldsmith, G. R., Schmid, L., Gessler, A., Saurer, M. and Siegwolf, R. T. W.: The effect of $^{18}$ O-labelled water vapour on the oxygen isotope ratio of water and assimilates in plants at high humidity, New Phytol., 217(1), 105–116, doi:10.1111/nph.14788, 2018.

Libby, L. M., Pandolfi, L. J., Payton, P. H., Marshall, J., Becker, B. and Giertz-Sienbenlist, V.: Isotopic tree thermometers, Nature, 261(5558), 284–288, doi:10.1038/261284a0, 1976.

Lister, D. H. and Jones, P. D.: Long-term temperature and precipitation records from the Falkland Islands, Int. J. Climatol., 35(7), 1224–1231, doi:10.1002/joc.4049, 2015.

Liu, H. T., Schäufele, R., Gong, X. Y. and Schnyder, H.: The δ18O and δ2H of water in the leaf growth-and-differentiation zone of grasses is close to source water in both humid and dry atmospheres, New Phytol., 214(4), 1423–1431, doi:10.1111/nph.14549, 2017.

Ménot, G. and Burns, S. J.: Carbon isotopes in ombrogenic peat bog plants as climatic indicators: calibration from an altitudinal transect in Switzerland, Org. Geochem., 32(2), 233–245, doi:10.1016/S0146-6380(00)00170-4, 2001.

Moreno-Gutiérrez, C., Dawson, T. E., Nicolás, E. and Querejeta, J. I.: Isotopes reveal contrasting water use strategies among coexisting plant species in a Mediterranean ecosystem, New Phytol., 196(2), 489–496, doi:10.1111/j.1469-8137.2012.04276.x, 2012.

Payne, R. J., Ring-Hrubesh, F., Rush, G., Sloan, T. J., Evans, C. D. and Mauquoy, D.: Peatland initiation and carbon accumulation in the Falkland Islands, Quat. Sci. Rev., 212, 213–218, doi:10.1016/j.quascirev.2019.03.022, 2019.

Pendall, E., Markgraf, V., White, J. W. C., Dreier, M. and Kenny, R.: Multiproxy Record of Late Pleistocene–Holocene Climate and Vegetation Changes from a Peat Bog in Patagonia, Quat. Res., 55(2), 168–178, doi:10.1006/qres.2000.2206, 2001.

Roden, J. S. and Ehleringer, J. R.: Observations of Hydrogen and Oxygen Isotopes in Leaf Water Confirm the Craig-Gordon Model under Wide-Ranging Environmental Conditions, Plant Physiol., 120(4), 1165–1174, doi:10.1104/pp.120.4.1165, 1999.

Sala, O. E., Golluscio, R. A., Lauenroth, W. K. and Soriano, A.: Resource partitioning between shrubs and grasses in the Patagonian steppe, Oecologia, 81(4), 501–505, doi:10.1007/BF00378959, 1989.

Saurer, M., Aellen, K. and Siegwolf, R.: Correlating δ13C and δ18O in cellulose of trees, Plant Cell Environ., 20(12), 1543–1550, doi:10.1046/j.1365-3040.1997.d01-53.x, 1997.

Scheidegger, Y., Saurer, M., Bahn, M. and Siegwolf, R.: Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model, Oecologia, 125(3), 350–357, doi:10.1007/s004420000466, 2000.

Schwinning, S. and Ehleringer, J. R.: Water use trade-offs and optimal adaptations to pulse-driven arid ecosystems, J. Ecol., 89(3), 464–480, doi:10.1046/j.1365-2745.2001.00576.x, 2001.

Smith, R. I. L. and Clymo, R. S.: An extraordinary peat-forming community on the Falkland Islands, Nature, 309(5969), 617–620, doi:10.1038/309617a0, 1984.

Smith, R. I. L. and Prince, P. A.: The natural history of Beauchêne Island, Biol. J. Linn. Soc., 24(3), 233–283, doi:10.1111/j.1095-8312.1985.tb00374.x, 1985.

Stanworth, A. and Upson, R.: Minefield Restoration Trials Final Report., 2013.

Sternberg, L. D. S. L., Deniro, M. J. and Savidge, R. A.: Oxygen Isotope Exchange between Metabolites and Water during Biochemical Reactions Leading to Cellulose Synthesis, Plant Physiol., 82(2), 423–427, doi:10.1104/pp.82.2.423, 1986.

Strange, I. J., Parry, C. J., Parry, M. C. and Woods, R. W.: Tussac grass in the Falkland Islands, 1988.

Thompson, D. W. J. and Solomon, S.: Interpretation of Recent Southern Hemisphere Climate Change, Science, 296(5569), 895–899, doi:10.1126/science.1069270, 2002.

Turner, J. and Pendelbury, S. (eds. ): The International Antarctic Weather Forecasting Handbook, in Sixth Conference on Polar Meteorology and Oceanography, American Meteorological Society, Boston, MA, San Diego, CA., 2000.

Turney, C. S. M., Jones, R. T., Lister, D., Jones, P., Williams, A. N., Hogg, A., Thomas, Z. A., Compo, G. P., Yin, X., Fogwill, C. J., Palmer, J., Colwell, S., Allan, R. and Visbeck, M.: Anomalous mid-twentieth century atmospheric circulation change over the South Atlantic compared to the last 6000 years, Environ. Res. Lett., 11(6), 064009, doi:10.1088/1748-9326/11/6/064009, 2016.

Upson, R., Williams, J. J., Wilkinson, T. P., Clubbe, C. P., Maclean, I. M. D., McAdam, J. H. and Moat, J. F.: Potential Impacts of Climate Change on Native Plant Distributions in the Falkland Islands, PLOS ONE, 11(11), e0167026, doi:10.1371/journal.pone.0167026, 2016.

Villalba, R., Lara, A., Masiokas, M. H., Urrutia, R., Luckman, B. H., Marshall, G. J., Mundo, I. A., Christie, D. A., Cook, E. R., Neukom, R., Allen, K., Fenwick, P., Boninsegna, J. A., Srur, A. M., Morales, M. S., Araneo, D., Palmer, J. G., Cuq, E., Aravena, J. C., Holz, A. and LeQuesne, C.: Unusual Southern Hemisphere tree growth patterns induced by changes in the Southern Annular Mode, Nat. Geosci., 5(11), 793–798, doi:10.1038/ngeo1613, 2012.

Weimerskirch, H., Louzao, M., de Grissac, S. and Delord, K.: Changes in Wind Pattern Alter Albatross Distribution and Life-History Traits, Science, 335(6065), 211–214, doi:10.1126/science.1210270, 2012.

White, J. W. C., Ciais, P., Figge, R. A., Kenny, R. and Markgraf, V.: A high-resolution record of atmospheric CO 2 content from carbon isotopes in pet, Nature, 367(6459), 153–156, doi:10.1038/367153a0, 1994.

Willis, K. J., Bailey, R. M., Bhagwat, S. A. and Birks, H. J. B.: Biodiversity baselines, thresholds and resilience: testing predictions and assumptions using palaeoecological data, Trends Ecol. Evol., 25(10), 583–591, doi:10.1016/j.tree.2010.07.006, 2010.

Wilson, P., Clark, R., McAdam, J. H. and Cooper, E. A.: Soil erosion in the Falkland Islands: an assessment, Appl. Geogr., 13(4), 329–352, doi:10.1016/0143-6228(93)90036-Z, 1993.

Yakir, D.: Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates, Plant Cell Environ., 15(9), 1005–1020, doi:10.1111/j.1365-3040.1992.tb01652.x, 1992.

**Table 1.** Correlation coefficients (Pearson's r) of $\delta^{18}O$ and $\delta^{13}C$ in leaf and root cellulose between $\delta^{18}O$ in monthly composite precipitation, monthly average temperature, and humidity by site. Bold values indicate significant correlations >0.600 Pearson's r. Significance level is $p < 0.05$.

| | Site | *n* | $\delta^{13}C_{leaf}$ | $\delta^{18}O_{leaf}$ | $\delta^{18}O$ precipitation | Temp | Relative Humidity |
|---|---|---|---|---|---|---|---|
| $\delta^{18}O_{leaf}$ | All sites | 24 | **0.877 (<0.001)** | -- | -0.201 (0.346) | **0.889 (<0.001)** | **-0.877 (<0.001)** |
| | Bleaker Is. | 6 | **0.864 (0.026)** | -- | -0.058 (0.913) | **0.947 (0.004)** | **-0.939 (0.005)** |
| | Cape Dolphin | 6 | **0.990 (<0.001)** | -- | -0.357 (0.487) | **0.877 (0.021)** | **-0.979 (<0.001)** |
| | Surf Bay | 6 | 0.769 (0.074) | -- | -0.378 (0.460) | **0.952 (0.003)** | **-0.977 (<0.001)** |
| | West Point Is. | 6 | **0.971 (0.001)** | -- | -0.330 (0.523) | **0.977 (<0.001)** | **-0.900 (0.014)** |
| $\delta^{18}O_{root}$ | All sites | 14 | -- | 0.385 (0.174) | -0.302 (0.294) | 0.311 (0.279) | -0.217 (0.457) |
| | Bleaker Is. | 6 | -- | 0.243 (0.642) | 0.222 (0.672) | 0.219 (0.677) | -0.116 (0.827) |
| | Cape Dolphin | 6 | -- | 0.623 (0.186) | **-0.868 (0.025)** | 0.701 (0.120) | -0.694 (0.126) |
| | Surf Bay | -- | -- | -- | -- | -- | -- |
| | West Point Is. | 2 | -- | -- | -- | -- | -- |
| $\delta^{13}C_{leaf}$ | All sites | 24 | -- | **0.877 (<0.001)** | -- | **0.817 (<0.001)** | **-0.759 (<0.001)** |
| | Bleaker Is. | 6 | -- | **0.864 (0.026)** | -- | **0.843 (0.035)** | -0.688 (0.131) |
| | Cape Dolphin | 6 | -- | **0.990 (<0.001)** | -- | **0.849 (0.032)** | **-0.952 (0.003)** |
| | Surf Bay | 6 | -- | 0.769 (0.074) | -- | 0.780 (0.067) | **-0.819 (0.046)** |
| | West Point Is. | 6 | -- | **0.971 (0.001)** | -- | **0.977 (<0.001)** | **-0.816 (0.047)** |
| $\delta^{13}C_{root}$ | All sites | 14 | **0.724 (0.003)** | -- | -- | 0.492 (0.074) | -0.299 (0.300) |
| | Bleaker Is. | 6 | **0.832 (0.039)** | -- | -- | 0.561 (0.247) | -0.273 (0.601) |
| | Cape Dolphin | 6 | 0.570 (0.237) | -- | -- | 0.778 (0.068) | -0.718 (0.108) |
| | Surf Bay | -- | -- | -- | -- | -- | -- |
| | West Point Is. | 2 | -- | -- | -- | -- | -- |

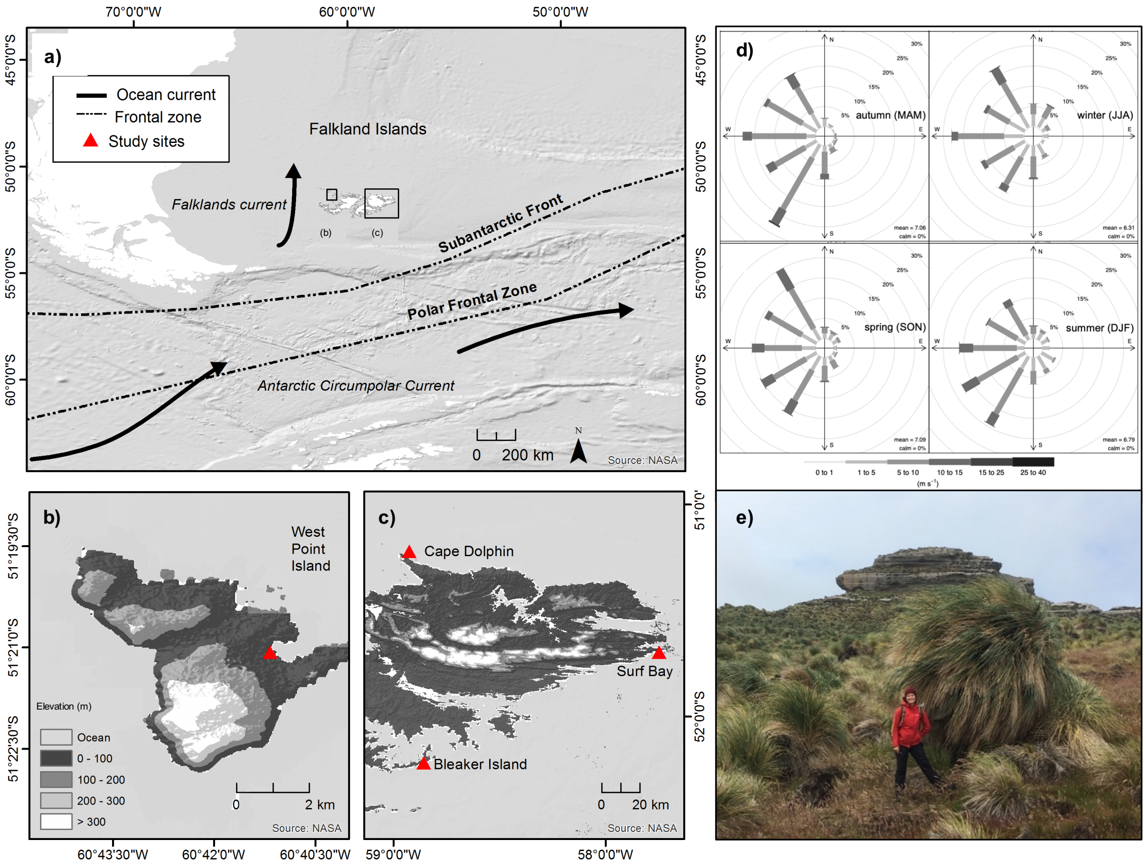


**Figure 1.** Study region. **a**) Map of the Falkland Islands and western South Atlantic Ocean with ocean currents (black arrows) and frontal zones (dashed lines). Study sites are shown in: **b)** West Point Island and **c)** Bleaker Island, Cape Dolphin, and Surf Bay. **d)** Distribution of wind speed (m s$^{-1}$) and the frequency of counts by wind direction (%) at Bleaker Island across four seasons autumn (MAM), winter (JJA), spring (SON), and summer (DJF), along with mean seasonal wind speed in m/s and % calm. Measurements logged at 15-minute intervals using Bleaker Island weather station (MetPak II) from September 2015-August 2016. Wind rose was constructed using the package *openair* in R version 3.1.0. **e)** A single large *Poa flabellata* pedestal made up of dead and living grass tillers growing on top of a decomposing pedestal at Cape Meredith, West Falkland, Falkland Islands.

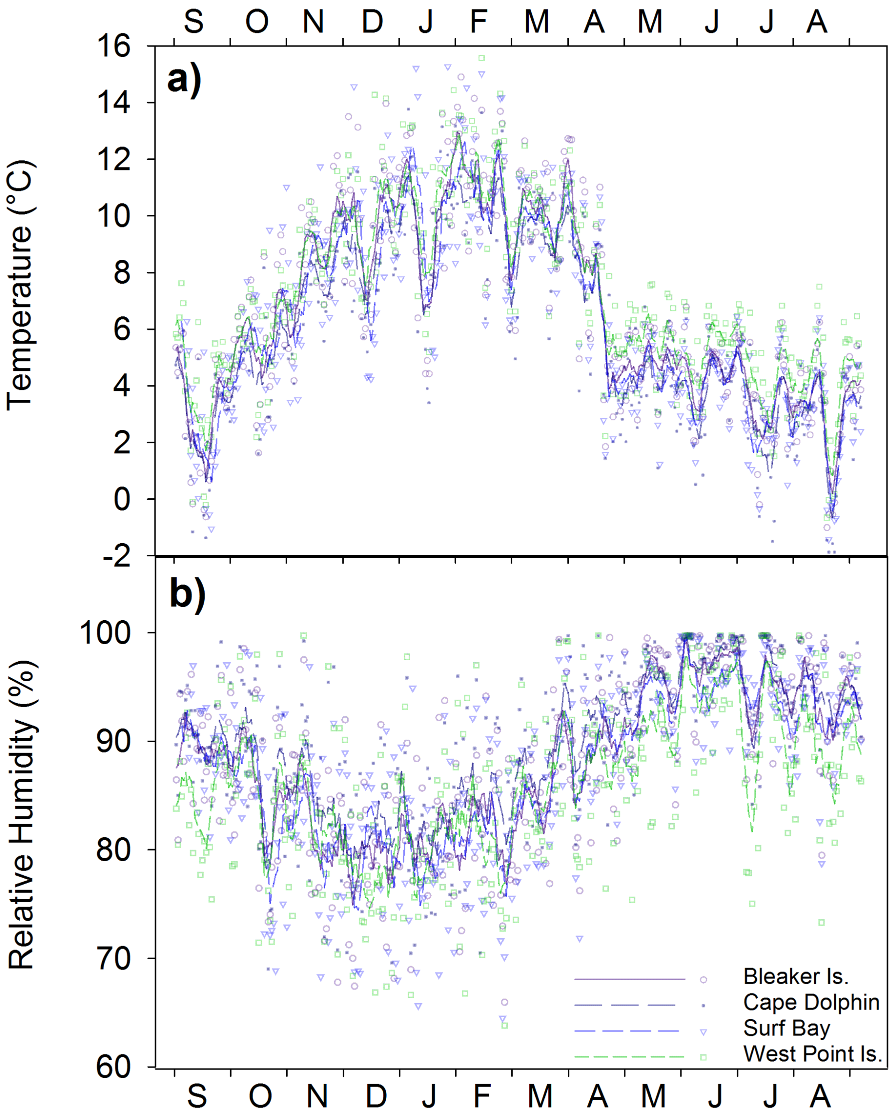


**Figure 2. a)** Daily average temperature (ºC) and **b)** relative humidity (%) from September 2015-August 2016 at the four study locations calculated from 2 hour measurements. Seven day running averages of daily average temperature and relative humidity are indicated by the lines for each study location.

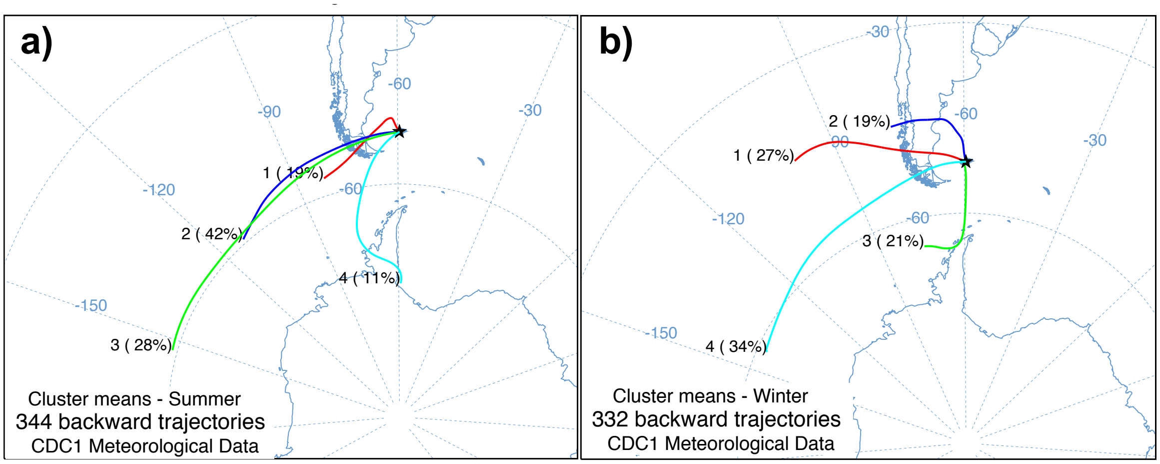


**Figure 3.** HYSPLIT seasonal cluster means of daily 5-day back trajectories from **a)** summer 2016 and **b)** winter 2015-16 at 51.79° S, -59.52° W with percentage of daily trajectories in each cluster and number of daily trajectories.

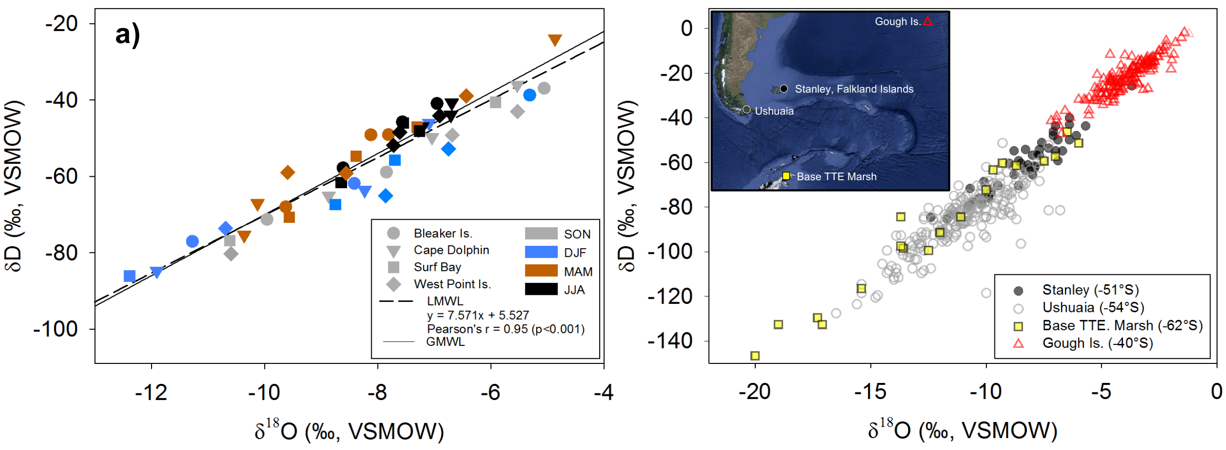


**Figure 4. a)** $\delta^{18}O$ and $\delta D$ (‰, VSMOW) isotopes in precipitation for each Falkland Islands location (symbol shape) during four seasons (symbol color). The constructed local meteoric water line (LMWL; $y = 7.571x + 5.527$; Pearson's correlation coefficient, $r = 0.95$, $p < 0.001$) is shown as a dashed line and global meteoric water line (GMWL: $\delta D = 8.0\ \delta^{18}O + 10$) is a solid line. **b)** Regional variation of $\delta^{18}O$ and in $\delta D$ in precipitation illustrating the variability in precipitation isotopes in the South Atlantic region. Corresponding locations of Global Network of Isotopes in Precipitation (GNIP) sites and potential sources of precipitation origin throughout the region over 30 years of data collection. Data from Stanley, Falkland Islands spans from 1962 to 1979; Ushuaia, Argentina from 1982 to 2002; Base TTE Marsh from 1990 to 1991; and Gough Island from 1961 to 2009; Data were downloaded from GNIP at https://nucleus.iaea.org/wiser. Map data: 2018 Google, SIO, NOAA, U.S. Navy, NGA, and GEBCO.

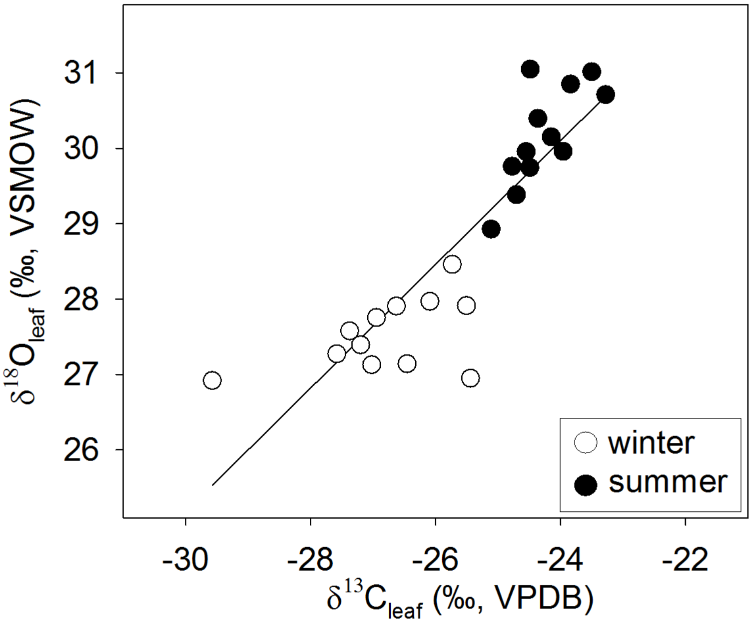
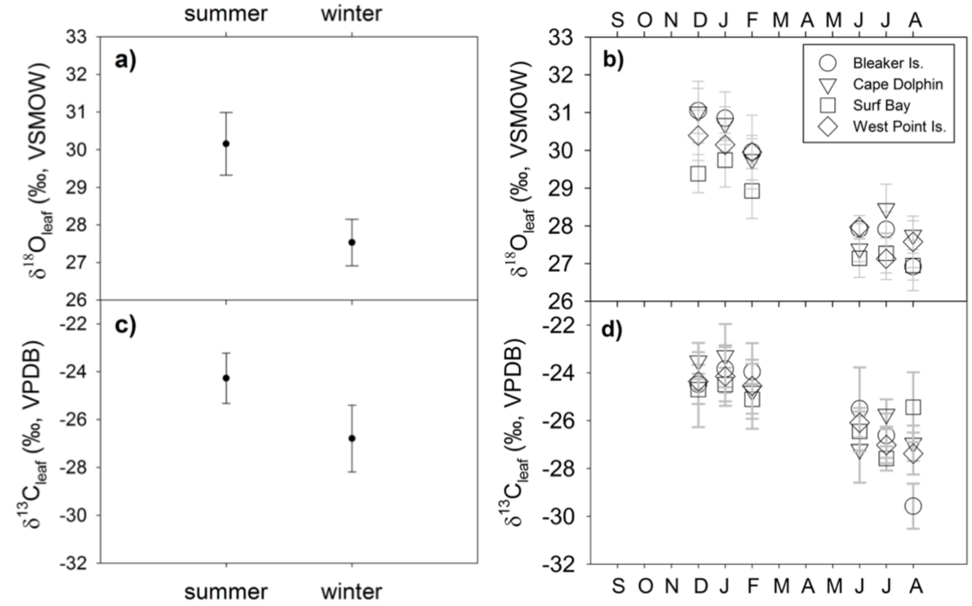


**Figure 5.** The relationship between average $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ (Pearson's correlation coefficient, $r = 0.877$, $p < 0.001$, $n = 24$). Open circles are average values for samples collected in winter, solid circles in summer.



535 **Figure 6.** $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ of *Poa flabellata*. **a)** $\delta^{18}O_{leaf}$ (‰) comparison (mean ± 1 SD) between summer (DJF) and winter (JJA) and, **b)** at four study sites over one year; **c)** $\delta^{13}C_{leaf}$ (‰) comparison (mean ± 1 SD) between summer and winter, and **d)** at four study locations over one year.

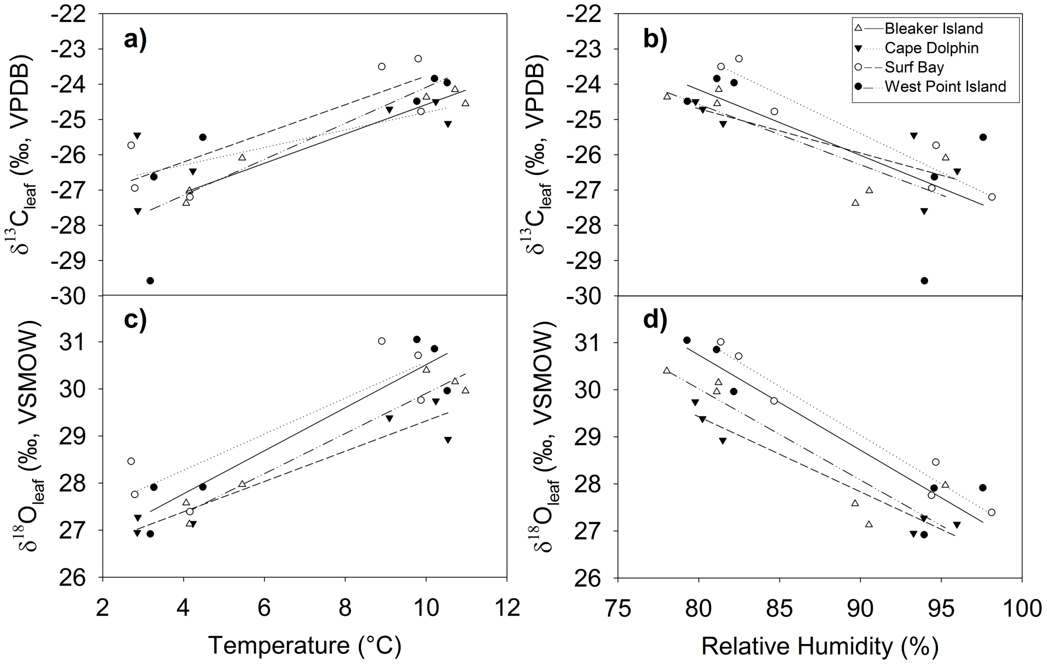


**Figure 7.** Relationship between $\delta^{13}C_{leaf}$ and $\delta^{18}O_{leaf}$ and temperature and relative humidity during winter and summer at four sites. Relationship between $\delta^{13}C_{leaf}$, **a)** temperature and **b)** humidity; Relationship between $\delta^{18}O_{leaf}$, **c)** temperature, and **d)** humidity. Use Table 1 for Pearson's r and p-values corresponding to correlations for each site.

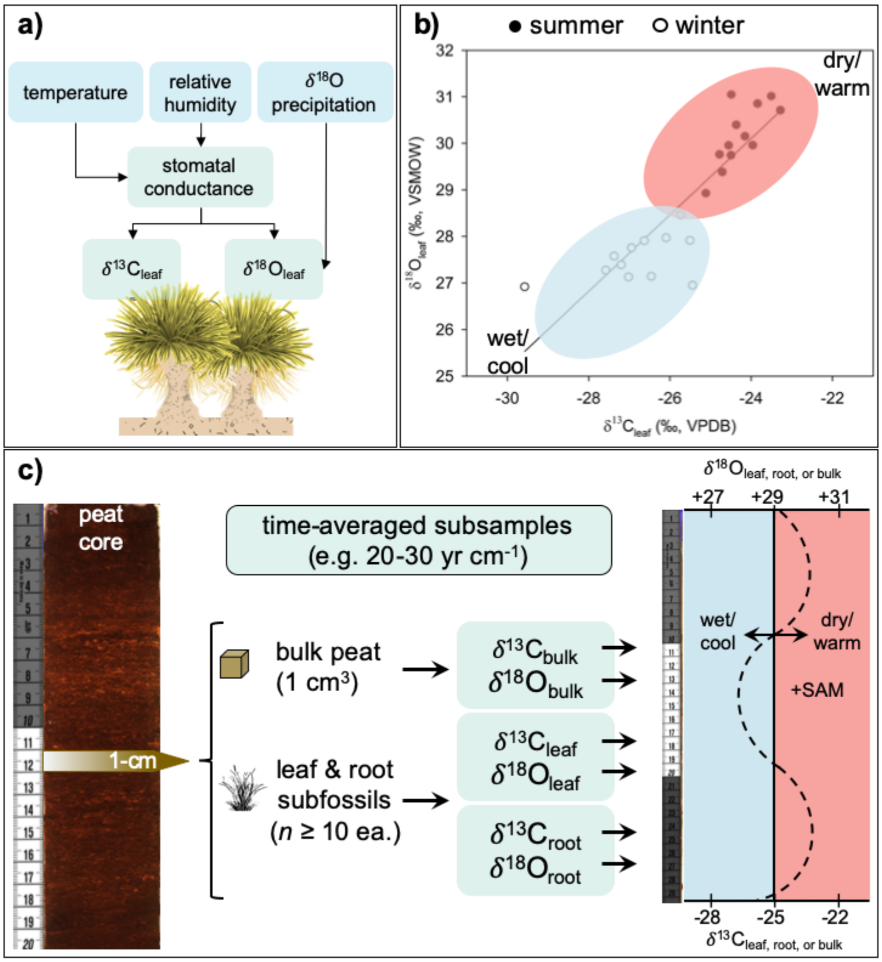


**Figure 8. a)** Conceptual model of how climate variation influences biomass $\delta^{13}$C and $\delta^{18}$O values in *P. flabellata* through effects on stomatal conductance and $\delta^{18}$O of precipitation. **b)** Seasonal shifts in leaf $\delta^{13}$C and $\delta^{18}$O, with open circles representing winter (cool/wet conditions) and closed circles representing summer (warm/dry conditions). **c)** Diagram of a proposed paleoclimate reconstruction workflow and interpretation of time-averaged (interannual) measurements of $\delta^{13}$C and $\delta^{18}$O in peat macrofossils.