

Temperature impact on the influence of penguin-derived nutrients and mosses on non-native grass in a simulated polar ecosystem

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Abstract

Human activity and climate change are increasing the spread of species across the planet, threatening biodiversity and ecosystem functions. Invasion engineers, such as birds, facilitate plant growth through manuring of soil, while native vegetation influences plant germination by creating suitable microhabitats which are especially valuable in cold and dry polar regions. Here we tested how penguin-derived nitrogen, several common Antarctic moss species and warming affect seed germination and growth of the non-native grass *Agrostis capillaris* under laboratory conditions. Experimental settings included a simulation of contemporary season-specific Antarctic light and temperature (2°C) conditions and a +5°C warming scenario. Mosses (*Andreaea depressinervis*, *A. regularis*, *Sanionia uncinata* and *Chorisodontium aciphyllum*) incorporated a range of nitrogen content and isotopic nitrogen signatures ($\delta^{15}\text{N}$) due to variation in sampling proximity to penguin colonies. Moss species greatly affected time to germination with consequences for further growth under the simulated Antarctic conditions. Grass seeds germinated 10 days earlier among *A. regularis* compared to *S. uncinata* and *C. aciphyllum* and 26 days earlier compared to *A. depressinervis*. Moss-specific effects are likely related to microclimatic differences within the moss canopy. Warming reduced this moss influence. Grass emerged on average 20 days earlier under warming, leading to increased leaf count (88%), plant height (112%) and biomass (145%). Positive correlations were identified between moss and grass nitrogen content ($r = 0.377$), grass biomass ($r = 0.332$) and height ($r = 0.742$) with stronger effects under the warming scenario. Transfer of nitrogen from moss to grass was confirmed by $\delta^{15}\text{N}$ ($r = 0.803$). Overall, the results suggest a shift from temperature-limited to N-limited growth of invasive plants under increased warming in the maritime Antarctic.

KEYWORDS

climate change, cryptogam, facilitation, invasion ecology, nitrogen, nitrogen isotopes

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1 | INTRODUCTION

Higher temperatures and increasing human travel are promoting the spread of non-native species into colder biomes (Bennett et al., 2015; Hughes et al., 2020) with potentially large impact on local communities and ecosystem processes (Pyšek et al., 2020). Seed germination is a crucial first hurdle non-native plants have to cross for establishment and this is determined by temperature and water conditions (Bokhorst et al., 2021; Thompson et al., 1977). Vegetation plays a key role in shaping underlying soil microhabitat conditions (Myers-Smith & Hik, 2013) and cryptogams take this role in many polar and alpine ecosystems (Blok et al., 2011; Soudzilovskaia et al., 2013). Mosses play an important role with regard to water availability as they retain water for long periods (Bokhorst et al., 2007; Oishi, 2018), thereby supporting high abundance of other biota and diversity within overall dry polar habitats (Convey et al., 2014). Moss influence on plant germination is typically largest in comparisons between presence versus absence of mosses (Drake et al., 2018; Jeschke & Kiehl, 2008) or moss depth (Stuiver et al., 2014; Zamfir, 2000). However, given the large morphological variation among moss growth forms and their impact on the microhabitats they create (Cornelissen et al., 2007; Hrbáček et al., 2020; Soudzilovskaia et al., 2013), species-specific effects seem likely but have rarely been tested. Low temperature and nutrient availability in polar ecosystems may further limit growth of both native and non-native plants (Vitousek & Howarth, 1991). Nutrient hotspots exist near bird colonies (Bokhorst et al., 2019; Otero et al., 2018) which may promote plant growth, although this may be temperature dependent (Wasley et al., 2006). Unravelling the complexity of the interacting biotic and abiotic factors that exist in polar ecosystems and how they impact on establishment of non-native species under climate warming is an important challenge that needs to be addressed in order to understand the trajectories polar ecosystems will follow in the near future.

Over 300 non-native plant taxa have been recorded in the Arctic (Wasowicz et al., 2020) and while this number is being approached on sub-Antarctic islands (Frenot et al., 2005) the number of non-native plants reaching the Antarctic Peninsula and continent is relatively low, primarily as a result of geographic isolation and low number of human visitors (Hughes & Convey, 2012). Non-native grasses have been recorded at various sites in the maritime Antarctic since the 1930s and their distribution was initially limited to the immediate vicinity of human structures (Longton, 1966; Smith, 1996), or as accidental transfers associated with early deliberate transplant experiments (Corte, 1961; Edwards, 1979). However, at Point Thomas on King George Island (South Shetland Islands), *Poa annua* L. has been spreading among the native moss and fellfield vegetation in recent years (Galera et al., 2019; Olech & Chwedorzewska, 2011). Understanding if and how native mosses will affect the spread of non-native species will help to identify which habitats are most likely to be affected by non-native species. Antarctic cushion-forming mosses (e.g. members of the widespread genera *Andreaea*, *Schistidium* and *Bryum* and associated species) typically form shallow cushions and experience large daily and seasonal variations in

temperature and water availability. The more extensive turf- (e.g. *Polytrichum strictum*-*Chorisodontium aciphyllum*) and carpet-forming (e.g. *Sanionia*-*Warnstorfia* associations) mosses experience, through their thicker and continuous mats, less variable diurnal and seasonal microclimatic conditions (Davey & Rothery, 1996; Fenton & Smith, 1982; Gimingham et al., 1971; Stanton et al., 2014). Carpet- and turf-forming mosses may, therefore, provide more suitable habitats for non-native plants than cushion-forming mosses in cold and dry polar ecosystems. Conversely, the densely packed shoots of turf-forming mosses may also prevent germination.

High nutrient availability increases the growth of non-native species across various regions on Earth (Brooks, 2003; Dawson et al., 2012; Flores-Moreno et al., 2016; He et al., 2011; Kołodziejek, 2019; Littschwager et al., 2010; Liu et al., 2018; Liu & Kleunen, 2017; Menz & Kettenring, 2013; Ross et al., 2011; Vasquez et al., 2008). However, nutrient availability is typically very limited in terrestrial Antarctica (Ball et al., 2018; Engelen et al., 2008) other than in the proximity of concentrated sources such as penguin colonies, seal aggregations and nesting birds (Ball et al., 2015; Bokhorst et al., 2019; Cocks et al., 1998; Erskine et al., 1998; Wasley et al., 2012; Zhu et al., 2014). Such ecosystem engineering by mammals and birds on nutrient availability for plants has been clearly recognized in polar ecosystems (Ball et al., 2015; Bokhorst et al., 2019; Gharajehdaghpour et al., 2016; Hilderbrand et al., 1999; McKendrick et al., 1980; Zwolicki et al., 2016), but whether this affects the establishment and growth of non-native species is unknown. Laboratory studies of the non-native grass *Poa annua* have indicated that it benefits from additional nutrients (Cavieres et al., 2018), suggesting that nutrient input by penguins and seals to Antarctic terrestrial ecosystems may promote the success of this non-native species.

The overall aim of this study is to identify how nutrients, different mosses and warming may affect the success of non-native plants in polar regions. Seed germination and subsequent growth of the non-native grass *Agrostis capillaris* L., which has already invaded several sub-Antarctic islands (Pertierra et al., 2016), was quantified in the laboratory under simulated contemporary Antarctic (mean summer season temperature of 2°C) and predicted warming conditions (7°C) in the presence of four different and common Antarctic moss species. The selected mosses were *Andreaea depressinervis* Cardot, *A. regularis* Müll. Hal. (cushion-forming), *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth (turf-forming) and *Sanionia uncinata* (Hedw.) Loeske (carpet-forming). The mosses were collected along natural nitrogen gradients (0.2–4.3%) resulting from distance from penguin colonies, allowing us to test for nitrogen effects on the growth of *A. capillaris* within each moss species. As the nitrogen gradients are associated with large differences in nitrogen isotopic signature $\delta^{15}\text{N}$ (Bokhorst et al., 2019) we also tested whether this isotopic signature is reflected in the tissue of *A. capillaris*. We hypothesized that (a) warming will accelerate germination and allow for more growth of *A. capillaris* across all moss species and irrespective of moss nitrogen content, considering the temperature constraints on biological activity in Antarctica (Convey et al., 2014); (b) carpet- and turf-forming mosses will facilitate more rapid germination and greater growth of

A. capillaris than cushion-forming mosses; (c) growth of *A. capillaris* will be enhanced by ornithogenic nitrogen input; and (d) the $\delta^{15}\text{N}$ of the various mosses will be reflected in that of *A. capillaris* as nutrient limitation will not allow for selection against heavier isotopes (Makarov, 2009). By addressing this set of hypotheses we aimed to identify the relative roles of temperature, nutrients and moss identity on plant growth in a polar ecosystem.

2 | METHODS

To test whether the growth of the non-native grass *A. capillaris* would benefit from nitrogen input by penguins we conducted a climate chamber experiment where we simulated Antarctic growing season conditions and grew the grass in different moss substrates that had been sampled at increasing distance from penguin colonies. Grasses are considered the most likely plant group to establish in Antarctica (Hughes et al., 2020), and *A. capillaris* has already invaded sub-Antarctic islands where it affects local biodiversity (Gremmen et al., 1998) and, therefore, can be considered a high threat to native maritime Antarctic ecosystems. The experiment ran for two growing seasons (140 days) with an intervening 6-month simulated winter period (-5°C in darkness). The climate chamber (THEBO Horeca) with RIVA Cold refrigeration units (Rivacold srl–Vallefoglia, Italy), was set to 2°C , based on mean growing season soil surface temperatures measured at Signy Island ($60^{\circ}\text{S } 45^{\circ}\text{W}$) and Anchorage Island ($67^{\circ}\text{S } 68^{\circ}\text{W}$) (Bokhorst et al., 2008). Diurnal variation in substrate temperature and light conditions were adapted every 4 weeks to mimic the seasonal variation in field microclimate conditions (Figure 1)

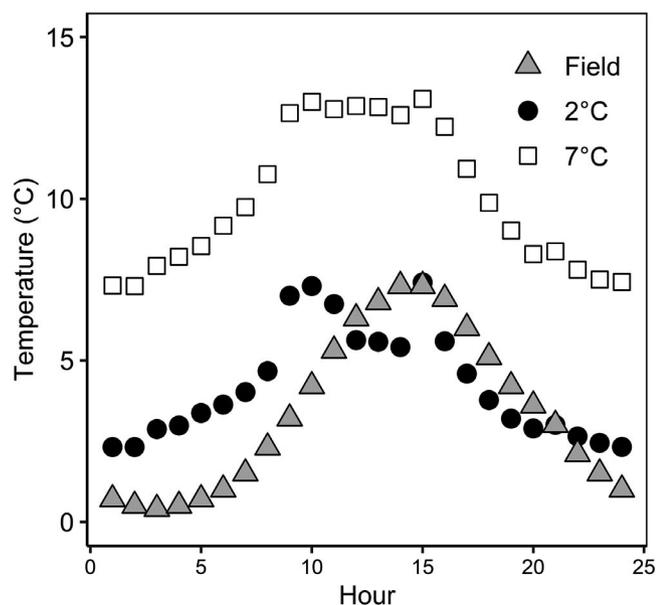


FIGURE 1 Diurnal temperature patterns. Soil surface diurnal temperature variation at Anchorage Island (67°S) (Field) during January and in the two experimental climate chambers simulating field conditions (2°C) and a 'business as usual' climate warming scenario (7°C)

(Bokhorst et al., 2007, 2008) by using light emitting diode (LED) lamps (Hortilight Sunfactor 270; 405 W). Relative humidity was kept between 60 and 90%, similar to field conditions, by placing a water bath in the climate chamber. Relative humidity and air temperature were recorded at hourly intervals (HOBO U23 Pro v2, Bourne, MA, USA) in both climate chambers. To quantify impacts of climate warming on the time required for germination and growth, a parallel climate chamber was run at 7°C . The 5°C temperature increase reflects the Representative Concentration Pathway (RCP) 8.5 global climate warming scenario (IPCC, 2013), which is appropriate given that the northern Antarctic Peninsula region already warmed by $\sim 3^{\circ}\text{C}$ in the second half of the 20th century and a warming trend of $\sim 0.5^{\circ}\text{C}/\text{decade}$ has been reported and is predicted for the remainder of the 21 century (Bracegirdle et al., 2019; Turner et al., 2014). Full details of the experimental climate simulation are described by Bokhorst et al. (2021).

The mosses used as growth substrate were sampled from Signy Island ($60^{\circ} 43' \text{S}$, $45^{\circ} 36' \text{W}$) and Byers Peninsula (Livingston Island; $62^{\circ} 39' \text{S}$, $61^{\circ} 05' \text{W}$) during the 2015/16 and 2016/17 austral summers respectively. Mosses were collected along transects starting at penguin colonies and running inland until reaching glacier edges or vegetation was no longer present. Intact moss plugs were collected by inserting a 50 ml plastic tube (28 mm diameter) into the top 5 cm of the moss layer, or shallower if moss depth was less. Tubes were sealed and stored frozen (-20°C) during transport to Europe. This sampling regime ensured that there was a range of nitrogen contents (0.19–4.26%) and $\delta^{15}\text{N}$ (-8.21 – 13.06‰) across the 124 sampled mosses (Table 1). Moss nitrogen and $\delta^{15}\text{N}$ was quantified on additional moss samples collected close to ($<0.5 \text{ m}$) each sampling location for a separate study (Bokhorst et al., 2019). We assumed that the nitrogen and $\delta^{15}\text{N}$ contents of the analysed moss samples were representative of the moss plugs used in this experiment due to the close proximity of sampling and the strong nitrogen gradients that exist along the transects. Sample size and nitrogen content were unequal between moss species because sampling distance to penguin colonies was used as a proxy for moss nitrogen content and uneven spatial distribution of mosses across sampling locations created additional variation. This precluded testing for interactions between species and nitrogen effects. Nevertheless, all species covered a range of nitrogen contents which allowed us to assess the impact of moss-associated nitrogen on plant growth across the whole data set and within each moss species.

Collected mosses differed between islands and transect sites, with the dark brown cushion-forming *Andreaea depressinervis*, and pale green turf-forming *Chorisodontium aciphyllum* collected from Signy Island and dark brown cushion-forming *A. regularis* and green carpet-forming *Sanionia uncinata* from Byers Peninsula. We tried to ensure that the field density of moss shoots remained intact when the mosses were sampled, as this may have consequences for plant growth. The moss plugs, with any decaying material beneath, were placed in plastic tubes (50 ml) filled approximately two thirds with plaster of Paris to ensure that the living surface moss layer was just beneath the top of each tube. Seeds of *A. capillaris* (Cruydtboek.nl;

Location	Species	Mean (SE)	2°C	7°C	(n)
Nitrogen (%)					
Signy Isl.	<i>A. depressinervis</i>	0.89 (0.10) ^b	0.93 (0.17)	0.85 (0.12)	29
Byers Pen	<i>A. regularis</i>	1.45 (0.13) ^a	1.48 (0.15)	1.42 (0.15)	50
Signy Isl.	<i>C. aciphyllum</i>	0.70 (0.08) ^b	0.62 (0.11)	0.77 (0.15)	20
Byers Pen	<i>S. uncinata</i>	0.96 (0.13) ^{ab}	0.92 (0.17)	1.00 (0.20)	25
$\delta^{15}\text{N}$ (‰)					
Signy Isl.	<i>A. depressinervis</i>	-2.72 (0.86) ^a	-1.71 (1.45)	-3.99 (0.49)	18
Byers Pen	<i>A. regularis</i>	1.73 (0.71) ^b	1.38 (0.94)	2.08 (1.09)	44
Signy Isl.	<i>C. aciphyllum</i>	1.92 (0.98) ^b	2.27 (0.88)	1.83 (1.23)	10
Byers Pen	<i>S. uncinata</i>	4.60 (1.76) ^b	7.93 (2.47)	1.82 (1.99)	11
Moss depth (mm)					
Signy Isl.	<i>A. depressinervis</i>	34.8 (1.6) ^{ab}	33.1 (1.9)	36.4 (2.6)	27
Byers Pen	<i>A. regularis</i>	29.5 (0.9) ^a	28.0 (1.3)	31.0 (1.1)	47
Signy Isl.	<i>C. aciphyllum</i>	40.9 (4.2) ^b	37.5 (4.3)	43.9 (7.9)	17
Byers Pen	<i>S. uncinata</i>	31.1 (1.4) ^{ab}	29.6 (1.5)	33.3 (2.9)	22

TABLE 1 Mean of moss nitrogen content, isotope signature and depth used in the experiment under simulated Antarctic climate conditions (2°C) and a climate warming scenario (7°C). Different letters within a column indicate significant (Tukey HSD, $p < 0.05$) differences between species. There were no species \times temperature effects. Note that for $\delta^{15}\text{N}$ we only show the moss values where sufficient grass biomass grew for isotope analyses. *Andreaea* spp. are cushion-forming mosses, *C. aciphyllum* is a turf-forming moss and *S. uncinata* is a carpet-forming moss

200 seeds by weight; 120 mg) were added to each tube and these were placed in a dark climate chamber at 2°C for cold stratification for 4 weeks. The moss layer was moistened using tap water *via* a plant sprayer once each week. After stratification, the tubes were divided between two climate chambers at the two (mean) temperatures of 2°C ($n = 62$) and 7°C ($n = 62$) ensuring that all species and N content were equally divided across the temperature regimes (Table 1). To ensure that the mosses remained moist during the experiment, the tubes were wetted by a plant sprayer using tap water twice each week. Water availability was therefore near unlimited for seedling growth but could have differed between moss species due to differences in evapotranspiration rates (Gimingham & Smith, 1971) between watering events. We did not quantify water loss rates during the growth experiment and any potential species effects on plant germination may, therefore, be in part a reflection of the moss morphological impacts on evapotranspiration. To avoid effects of placement within each chamber, tube positions were randomly repositioned every week and all tubes were moved between chambers every month to avoid any systematic 'chamber effect' throughout the experiment. The temperature settings were readjusted when plants were moved between chambers.

2.1 | Biological measurements

From the onset of spring, when lights came on, we noted the number of days required for the first seedling to germinate in each experimental tube at 3–4 day intervals. From this we calculated germination time (days) of *A. capillaris*. We counted the total number of leaves and maximum plant height within each experimental tube at the end of the first and second growing seasons. In addition, we harvested all living *A. capillaris* plants at the end of the second growing season, counted the number of plants per tube and quantified total biomass after oven drying (48 h at 70°C). From this we calculated

the number of leaves per plant and mean plant biomass. Nitrogen content and $\delta^{15}\text{N}$ of *A. capillaris* were quantified by dry combustion in an NC 2500 elemental analyzer (Carlo Erba) coupled with a Deltaplus continuous-flow isotope ratio mass spectrometer (Thermo Finnigan). Due to the low biomass of some grass samples only 83 (of the 124) could be analysed for $\delta^{15}\text{N}$. We measured moss depth with a ruler for each tube. However, as this was done after destructive harvesting for *A. capillaris*, 11 samples were too damaged for accurate depth measurements. To quantify species-specific effects on moss temperature we measured surface moss temperatures, using temperature loggers (I-Button, Maxim integrated) placed just below the moss surface, for a 13-day period under simulated summer light conditions at 2°C and 7°C (with total $n = 4$ loggers per species). Moss evapotranspiration rates were quantified during a day–night cycle where mass loss of water saturated samples ($n = 5$ per species) was measured at regular intervals. Temperature and evaporation measurements were made on remaining moss samples after the growth experiments.

2.2 | Data analyses

Factorial ANOVA was used to compare the starting N-content, $\delta^{15}\text{N}$ and moss depth between moss species and their allocation between 2°C and 7°C treatments. Moss-specific effects on temperature and evapotranspiration rates at 2°C and 7°C were compared using ANOVA. We used a factorial ANOVA with moss species, temperature, N-content and moss depth as explanatory variables to explain time to germination, plant height, number of leaves (first growing season only), total plant count, number of leaves per plant, mean plant biomass and total biomass of *A. capillaris* (second growing season). Moss depth did not emerge as a significant explanatory variable and there were few significant interaction terms, so only the main significant factors and their interactions are presented. In the

case of significant species \times temperature interactions, we quantified the difference between 2°C and 7°C for each moss species, thereby ranking experimental unit values and analysing these through one-way ANOVA. Correlations were calculated between plant variables and moss-specific variables (N-content, $\delta^{15}\text{N}$, moss depth). To identify the most important predictors (temperature, moss nitrogen, moss depth and time to germination) for grass height, leaf counts and biomass we performed stepwise regression (in both directions) using the stepAIC function in the MASS package (Venables & Ripley, 2002). We then calculated the relative importance of the most important predictors for the grass variables for each moss species separately using the relaimpo package (Grömping, 2006). Normality of data distribution was visually inspected through q-q plots and data were log-transformed to improve homogeneity of variances. Statistical analyses were performed using R (R-Core-Team, 2021).

3 | RESULTS

3.1 | Moss species differences

Mean nitrogen content of *A. regularis* (1.45%) was greatest of all species (Table 1). The range in $\delta^{15}\text{N}$ signature was greatest among both *Andreaea* species and least for *C. aciphyllum*. Mean isotopic $\delta^{15}\text{N}$ signature was least for *A. depressinervis*. Mean moss depth was greatest for *C. aciphyllum* and overall moss depth was 14% greater for the samples kept at 7°C compared to 2°C. Moss temperature and evapotranspiration rates were higher at 7°C but did not differ between species (Table S1).

3.2 | Emergence

Seedlings germinated in 82% of the tubes at 2°C and 98% at 7°C during the first growing season and further growth was observed in 91% and 85% of tubes during the second season respectively. There were on average 10 grass plants per experimental tube at

the end of the second simulated growing season and this was unaffected by moss species, temperature or nitrogen content (Table 2). Seedlings germinated first among *A. regularis* followed by *C. aciphyllum*, *S. uncinata* and *A. depressinervis*, resulting in, respectively, 75, 66, 64 and 49 days remaining for growth during the first growing season (Figure 2a). During the second growing season, grass shoots emerged first among *A. regularis* and last among *S. uncinata*, with *A. depressinervis* and *C. aciphyllum* being intermediate (Figure 2b).

Grass emergence took on average 20 days longer at 2°C than 7°C during both growing seasons (Table 2, Figure 2). At 2°C, grass emerged earliest among *A. regularis* while there were no differences in emergence time for the other mosses. This pattern was repeated during the second growing season. Grass emergence was 24 days delayed when growing among *A. depressinervis* at 7°C during the first growing season, while no moss effects were detected between the other species or during the second growing season at this temperature. These differences resulted in large species-specific variation in response to warming (Figure 2). Nitrogen content did not affect time to grass emergence (Table 2).

3.3 | Plant production

Grass grew tallest among *A. regularis* and shortest among *A. depressinervis* during the first growing season (Figure 2c) and shortest among *S. uncinata* during the second growing season (Figure 2d). Warming increased grass height during both the first (112%) and second (67%) seasons (Table 2, Figure 2c,d). Grass height was positively correlated ($r = 0.742$, $p < 0.001$) with moss N-content, most strongly so under the warming scenario (Figure 3a, Figure S1 and S2).

Total grass leaf count was 81% higher when grown among *A. regularis* compared to *A. depressinervis* during the first growing season. *Post hoc* testing did not indicate further moss species differences during either simulated growing season. Total number of leaves was 88% higher at 7°C than 2°C during the second simulated growing season. Mean leaf count per plant was highest (Tukey HSD $p < 0.05$) among *A. regularis* compared to *A. depressinervis* but no

TABLE 2 ANOVA results (F -values) of *Agrostis capillaris* emergence time, plant length, leaf count, number of emerged plants and biomass when grown under simulated Antarctic climate conditions (2°C) and a climate warming scenario (7°C) during two consecutive summer seasons; first (1) and second (2) grown among four different moss species (SP: *Andreaea depressinervis*, *A. regularis*, *Chorisodontium aciphyllum* and *Sanionia uncinata*) each with varying nitrogen (N) content. There were no main effects for moss depth and there were no meaningful (three-way) interactions

		Species	Temperature	Nitrogen	SP \times T	T \times N
Emergence time	(1)	13.4***	50.2***	0.0	4.5**	1.3
	(2)	10.6***	55.7***	0.8	5.7**	0.6
Plant height	(1)	2.6 ⁺	51.6***	16.6***	1.1	12.7***
	(2)	4.2**	35.5***	25.3***	1.6	6.1 ⁺
No. leaves	(1)	4.3**	1.8	3.0 ⁺	2.9 ⁺	0.5
	(2)	2.8 ⁺	20.1***	2.2	1.1	0.1
No. plants	(2)	2.2 ⁺	2.2	0.1	0.8	0.5
No. leaves/plant	(2)	3.9 ⁺	96.0***	13.6***	0.8	2.1
Mean plant biomass	(2)	4.6**	26.5***	9.0**	0.5	12.5***
Total biomass	(2)	5.3**	21.8***	5.3 ⁺	0.2	3.3

⁺ $p < 0.1$;

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

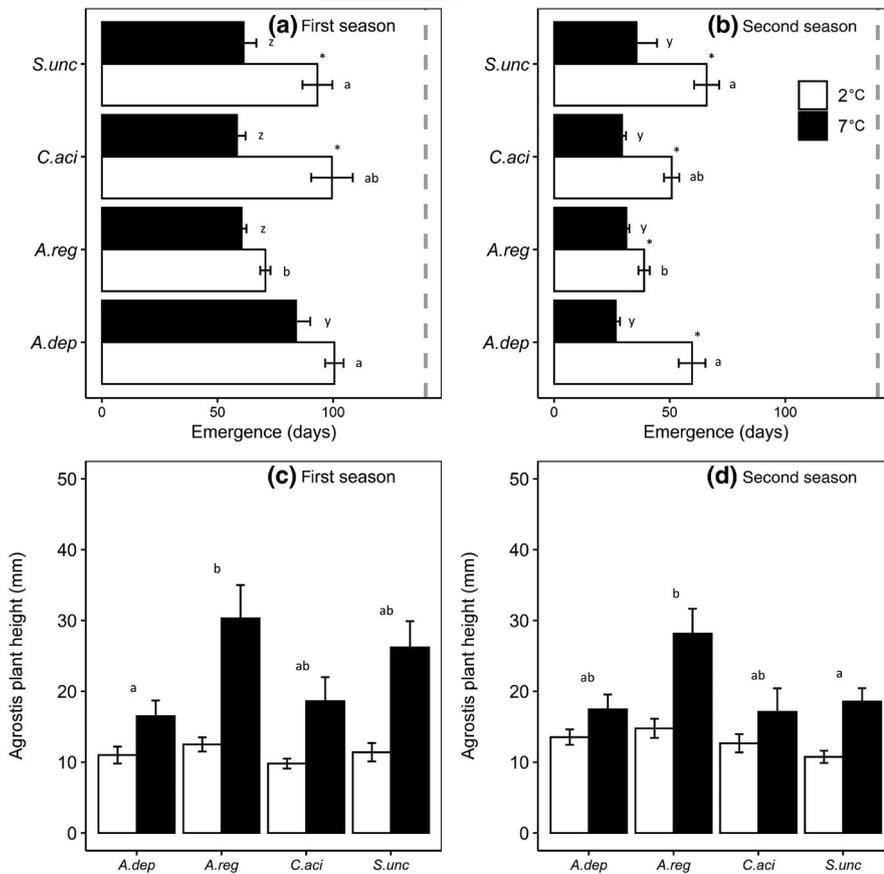


FIGURE 2 Emergence and growth of an invasive plant. Emergence time and plant height of *Agrostis capillaris* grown among different moss species under simulated Antarctic climate conditions (2°C) and a climate warming scenario (7°C) during two consecutive growing seasons. The vertical dashed line represents the end of the growing season (day 140). Bars are means of $n = 6-26$, with SE as error. Bars with the same letters do not significantly differ (Tukey HSD) considering the mean species difference for c and d and at specific temperatures for a and b. (***) denotes significant temperature effect. ANOVA results are shown in Table 2. *A. dep* = *Andreaea depressinervis*, *A. reg* = *Andreaea regularis*, *C. aci* = *Chorisodontium aciphyllum*, *S. unc* = *Sanionia uncinata*

differences were found with the other moss species (Table 2). Mean leaf count was 69% higher at 7°C than 2°C and positively correlated with moss N-content (Figure 3b).

Grass biomass was highest when grown among both *Andreaea* spp. compared to *S. uncinata* and *C. aciphyllum*. This pattern was also found for the mean biomass among moss species except that the effect of moss-N on mean biomass was stronger at 7°C than 2°C (Figure 3c). Total biomass was 145% higher when grown at 7°C compared to 2°C (Table 2) and was positively correlated ($r = 0.332$, $p < 0.001$) with moss N-content across the entire data set (Figure 3d). Grass nitrogen was positively correlated ($r = 0.377$) with that of the moss (Figure 4) with mean N-values higher ($F_{1,68} = 22.9$, $p < 0.001$) at 2°C ($1.86 \pm 0.12\%$) compared to 7°C ($1.31 \pm 0.05\%$).

Later grass emergence resulted in lower leaf production across all moss species (Figure S3, Table 3). Temperature was the main driver of leaf count per plant (Table 3). Grass height was primarily affected by emergence time and temperature when growing among *C. aciphyllum* and *S. uncinata* during the first growing season while temperature and moss-N appeared stronger drivers for grass height when growing among both *Andreaea* species (Table 3). However, during the second growing season, emergence time was the most important factor for grass height and biomass when growing among *A. depressinervis* and *S. uncinata* while temperature and nitrogen were dominant when growing among *A. regularis* and *C. aciphyllum*.

Grass $\delta^{15}\text{N}$ content was positively correlated ($r = 0.438$, $p < 0.001$) with that of the moss substrate but this was primarily found for the individuals grown among *A. regularis* ($r = 0.803$, $p < 0.001$, Figure 5). There was a positive trend ($r = 0.408$, $p = 0.083$) of grass $\delta^{15}\text{N}$ with that of *A. depressinervis* while no correlations were observed for grass $\delta^{15}\text{N}$ grown among *C. aciphyllum* or *S. uncinata*. The mean grass $\delta^{15}\text{N}$ signature differed when compared between these three moss species ($F_{2,38} = 4.3$, $p = 0.020$) with the lowest $\delta^{15}\text{N}$ values found when grown among *A. depressinervis* ($0.35 \pm 0.43\%$) followed by *C. aciphyllum* ($2.20 \pm 0.63\%$) and *S. uncinata* ($3.42 \pm 1.17\%$).

4 | DISCUSSION

The overall aim of this study was to better understand how mosses affect the success of non-native plants in polar regions. The data obtained clearly support the hypothesis that penguins may potentially act as invasion engineers through their manuring activity on land which can benefit the growth of non-native plants, similar to animal-activity effects on plant growth reported at lower latitudes (Farji-Brener et al., 2010; Zhang et al., 2013). However, this nitrogen effect was limited by temperature and there were large differences between moss species on grass seed germination, growth and nitrogen pathways. The successful establishment and growth of non-native vascular plants in polar regions will therefore result

FIGURE 3 Nitrogen and temperature impacts on plant size and biomass. Correlation of *Agrostis capillaris* plant height (a), number of leaves (b) mean plant biomass (c) and total biomass (d) with moss nitrogen content under simulated contemporary Antarctic climate conditions (grey; 2°C, n = 57) and a climate warming scenario (black; 7°C, n = 52) at the end of the second growing season. First growing season data are presented in Figure S2. Grey and black solid lines represent correlations at 2°C and 7°C respectively with r and p-values presented at the top of each panel. Dashed line shows correlation across entire data set. 95% confidence intervals are represented by grey shading

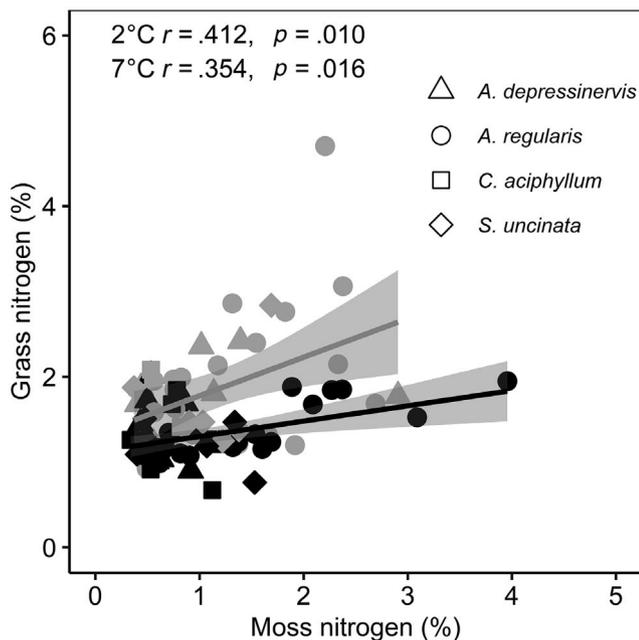
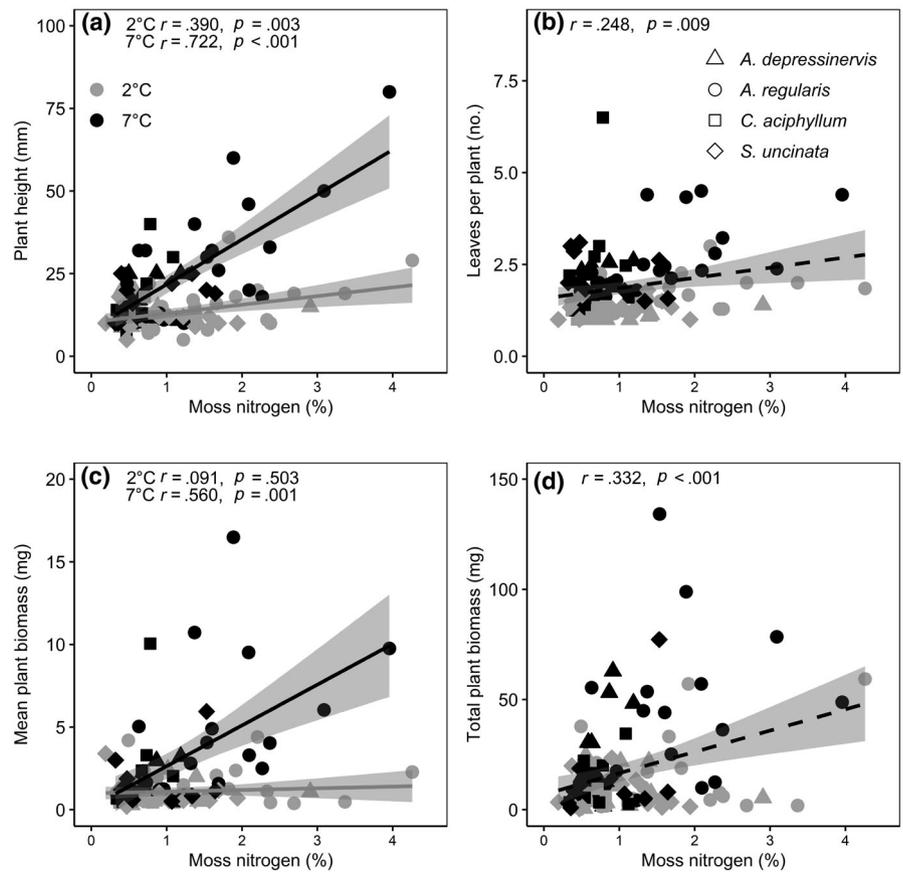


FIGURE 4 Nitrogen content of *Agrostis capillaris*. Correlation of *Agrostis capillaris* nitrogen content with that of the moss substrate under simulated contemporary Antarctic climate conditions (grey; 2°C, n = 38) and a climate warming scenario (black; 7°C, n = 47). Lines show correlation across both temperatures. Ninety-five per cent confidence intervals are represented by grey shading. Mean grass nitrogen content is higher at 2°C compared to 7°C ($F_{1,68} = 22.9$, $p < 0.001$) but there is no significant difference in r-values according to a Fisher-z test (0.299, $p = 0.765$; two tailed)

from interplay between invasion engineering, temperature and moss identity.

4.1 | Warming reduces moss species effects on vascular plant germination

There was support for our first hypothesis in that plants germinated earlier in response to warming, giving them a longer subsequent growth period. There were also clear differences in plant emergence between the different moss species, although these did not conform with our other hypothesis. Grass emerged most rapidly among the cushion-forming moss *A. regularis*, allowing about 75 days for subsequent growth, while this was reduced to 50 days for the other cushion-forming moss *A. depressinervis*, with intermediate values for the turf- and carpet-forming mosses. These emergence differences also affected plant size and biomass at the end of the growing season but again not consistently across all moss species. Moss influence on plant germination is often largest in comparisons between presence and removal of mosses (Drake et al., 2018; Jeschke & Kiehl, 2008) or moss depth (Stuiver et al., 2014; Zamfir, 2000), but our results clearly showed moss-species-specific effects and no detectable effects of moss depth. Soudzilovskaia et al. (2011) reported differences in the number of plant seedlings as a result of moss-species-specific effects on temperature and phenolic leachates but, contrary to our results, did not identify any effects on plant biomass. The main species-specific effects of that study were driven by liverworts, with

	First season				Second season			
	A. dep	A. reg	C. aci	S. unc	A. dep	A. reg	C. aci	S. unc
No. leaves								
Emergence	85	59	69	80	75	9	25	89
Temperature	13	34	27	14	24	71	13	10
Nitrogen	2	7	4	6	1	20	62	1
Variance explained	56%	33%	63%	67%	81%	25%	17%	71%
Leaves/plant								
Emergence					38	2	30	15
Temperature					60	67	54	77
Nitrogen					2	31	16	8
Variance explained					82%	59%	39%	57%
Plant height								
Emergence	18	17	46	63	70	2	12	67
Temperature	31	50	53	32	28	53	24	31
Nitrogen	51	33	1	5	2	45	64	2
Variance explained	35%	45%	35%	75%	22%	49%	25%	78%
Mean plant biomass								
Emergence					39	6	44	14
Temperature					37	61	44	83
Nitrogen					24	33	12	3
Variance explained					16%	33%	29%	18%
Biomass								
Emergence					76	16	43	87
Temperature					21	60	21	12
Nitrogen					3	24	36	1
Variance explained					59%	32%	30%	44%

Abbreviation: *A. dep*, *Andreaea depressinervis*, *A. reg*, *A. regularis*, *C. aci*, *Chorisodontium aciphyllum* and *S. unc*, *Sanionia uncinata*.

very few differences between the mosses tested. The moss-species-specific influences on grass germination were reduced at 7°C, suggesting that climate warming may reduce the importance of moss influence in the cold maritime Antarctic ecosystems.

Warming effects on grass germination differed greatly between moss species (11–49 days), due to different times to germination/emergence at 2°C. Temperature and water availability are the most likely factors behind these differences, but subsurface temperature and evapotranspiration rates did not differ between the tested moss species (Table S2). Possibly the moss species influence on seed germination plays out within the moss canopy, requiring carefully controlled conditions to measure temperature and evapotranspiration at micro-scales. Moss colour can affect leaflet temperature with darker colours absorbing more incoming radiation than lighter (Glime, 2017). This could provide an explanation as to why germination among the pale green moss *C. aciphyllum* was much higher at 7°C whereas the dark brown *A. regularis* may have warmed more under high light at 2°C and

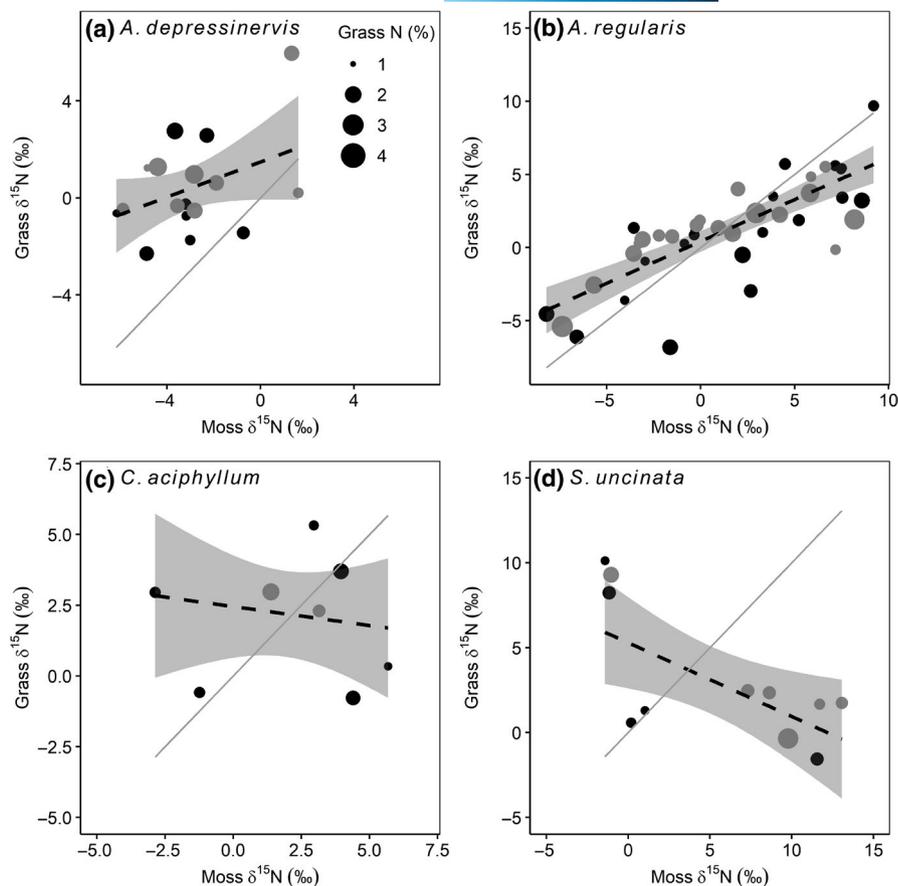
TABLE 3 Relative contribution of explanatory variables on grass growth. The total proportion of variance of grass leaf count, height and biomass explained by the most important explanatory variables, derived from stepwise regression

thereby reduced the 'time' (temperature sum) gap between the 2°C and 7°C treatment. Additional moss characteristics that may affect plant germination include morphological characteristics and chemical traits (Cornelissen et al., 2007; During et al., 1990; Serpe et al., 2006; Stoy et al., 2012; Tooren, 1990). Our data suggest that large inter-specific differences exist among the four common Antarctic moss species studied in their potential influence on plant germination, although the mechanism underlying this remains unclear. Considering that these moss species are widely distributed across other biomes (Hedenäs, 2011), it is reasonable to suggest that these species-specific differences may also affect plant growth in, for instance, Arctic and alpine ecosystems.

4.2 | Invasion engineering by penguins

Higher nitrogen content of moss was associated with increased plant height and biomass, as hypothesized. However, this influence

FIGURE 5 $\delta^{15}\text{N}$ of *Agrostis capillaris* grown among different mosses. Correlation of *Agrostis capillaris* $\delta^{15}\text{N}$ with that of the growth substrate moss sample (*Andreaea depressinervis*, *A. regularis*, *Chorisodontium aciphyllum* or *Sanionia uncinata*). Symbol size represents the nitrogen content (%) of the grass and symbol colour the temperature at which it grew (grey = 2°C and black = 7°C). Dashed regression line is used to depict the association of $\delta^{15}\text{N}$ between source (moss) and sink (*A. capillaris*); grey shading shows 95% confidence interval. Grey line represents the 1:1 ($y = x$) line of moss $\delta^{15}\text{N}$



of nitrogen was limited at the simulated contemporary Antarctic field conditions of 2°C, perhaps suggesting that low temperatures may be a reason why the Antarctic Peninsula lacks lush vegetation in the proximity of bird cliffs as can be found on sub-Antarctic islands and in the Arctic (Ellis, 2005; Zwolicki et al., 2016). The higher nitrogen content of grasses grown at 2°C compared to 7°C reflects a dilution factor resulting from the higher plant biomass under warming (Greenwood et al., 1990). Introduced non-native species are therefore unlikely to benefit from high nutrient loadings in the proximity of penguin colonies under the current climate. This was also found under laboratory conditions for the non-native *Poa annua* when competing with the native *Deschampsia antarctica* under a warming scenario and nitrogen additions (Cavieres et al., 2018). However, the current study used Antarctic substrate with nitrogen content that is naturally present in the field. Penguins and other marine vertebrates, such as seals, that transfer large amounts of nutrients from the ocean to land in Antarctica (Bokhorst et al., 2019; Lindeboom, 1984) may act as invasion engineers in synergy with climate warming for non-native plants, similar to ant and crab activity reported elsewhere (Farji-Brener et al., 2010; Zhang et al., 2013). Sites of animal activity that affect soil nutrient availability may therefore present some of the best opportunities for non-native plants to establish and require particular attention with respect to environmental protection and biosecurity measures.

4.3 | Nitrogen transfer from moss to grass depends on moss species

We used $\delta^{15}\text{N}$ signatures in an attempt to link the nitrogen source used by the grass with that of the mosses in the experiment. The underlying assumption was that little isotopic fractionation of $\delta^{15}\text{N}$ would take place when vascular plants grow among the nutrient-limited Antarctic mosses (Makarov, 2009). In addition, the general lack of mycorrhizal fungi in Antarctica to support nutrient scavenging by vascular plants (Hill et al., 2019) would limit further isotopic fractionation. This assumption was supported by the near 1:1 ratio of $\delta^{15}\text{N}$ signatures of the grass with that of *A. regularis*. The most likely nitrogen source was therefore direct leakage from moss shoots, a nutrient source which has also been reported in Arctic studies (Pouliot et al., 2009). However, the $\delta^{15}\text{N}$ signatures of the other three moss species studied here were poorly reflected in the grass, suggesting that those moss species are better at retaining nitrogen and/or isotopic fractionation through microbial activity had taken place (Makarov, 2009). The close to zero $\delta^{15}\text{N}$ signature of the grass when grown among *A. depressinervis* suggests N-fixation activity in the moss tissue which, although not reported in maritime Antarctic studies, has been reported in *Andreaea* sp. on sub-Antarctic Marion Island (Line, 1992). These data indicate that there is potentially large inter-specific variation in nitrogen cycling among Antarctic mosses, with important consequences for competition with vascular plants.

Climate change is rapidly affecting temperature and water regimes in polar ecosystems (Convey et al., 2014). The current findings indicate that Antarctic ecosystems may shift from temperature-limited to N-limited plant growth, as reported in Arctic regions (Shaver et al., 1980). Penguin colonies and other marine vertebrate aggregations will, therefore, likely become hotspots for the establishment of non-native plants. Mosses, through their control over water availability and temperature, exert an important influence on plant germination and growth, but not consistently between species and their effects are diminished by warming. The relative roles of temperature, water and nutrients in these Antarctic ecosystems illustrate a complex mixture of local biotic and abiotic factors, as also observed in the heterogeneous greening and browning of the Arctic (Myers-Smith et al., 2020). Predictions of climate change influence on future polar ecosystem change must take this local complexity into account.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

SB, PC and RA designed the experiment. SB and RvL carried out the experiment and performed the laboratory analyses. SB, PC, RvL and RA contributed to writing of the manuscript.

DATA AVAILABILITY STATEMENT

Data are archived and available at the Netherlands Polar Data Center (NPDC.nl) <https://npdc.nl/dataset/9c54448b-50fe-50bd-b36e-3952c80c5cfd>

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REFERENCES

- Ball, B. A., Adams, B. J., Barrett, J. E., Wall, D. H., & Virginia, R. A. (2018). Soil biological responses to C, N and P fertilization in a polar desert of Antarctica. *Soil Biology & Biochemistry*, 122, 7–18. <https://doi.org/10.1016/j.soilbio.2018.03.025>
- Ball, B. A., Tellez, C. R., & Virginia, R. A. (2015). Penguin activity influences soil biogeochemistry and soil respiration in rookeries on Ross Island, Antarctica. *Polar Biology*, 38, 1357–1368. <https://doi.org/10.1007/s00300-015-1699-7>
- Bennett, J. R., Shaw, J. D., Terauds, A., Smol, J. P., Aerts, R., Bergstrom, D. M., Blais, J. M., Cheung, W. W. L., Chown, S. L., Lea, M.-A., Nielsen, U. N., Pauly, D., Reimer, K. J., Riddle, M. J., Snape, I., Stark, J. S., Tulloch, V. J., & Possingham, H. P. (2015). Polar lessons learned: long-term management based on shared threats in Arctic and Antarctic environments. *Frontiers in Ecology and the Environment*, 13, 316–324. <https://doi.org/10.1890/140315>
- Blok, D., Heijmans, M., Schaepman-Strub, G., van Ruijven, J., Parmentier, F. J. W., Maximov, T. C., & Berendse, F. (2011). The cooling capacity of mosses: Controls on water and energy fluxes in a Siberian tundra site. *Ecosystems*, 14, 1055–1065. <https://doi.org/10.1007/s10021-011-9463-5>
- Bokhorst, S., Convey, P., & Aerts, R. (2019). Nitrogen inputs by marine vertebrates drive abundance and richness in Antarctic terrestrial ecosystems. *Current Biology*, 29, 1721–1727. <https://doi.org/10.1016/j.cub.2019.04.038>
- Bokhorst, S., Convey, P., Casanova-Katny, A., & Aerts, R. (2021). Warming impacts potential germination of non-native plants on the Antarctic Peninsula. *Communications Biology*, 4, 403. <https://doi.org/10.1038/s42003-021-01951-3>
- Bokhorst, S., Huiskes, A., Convey, P., & Aerts, R. (2007). The effect of environmental change on vascular plant and cryptogam communities from the Falkland Islands and the Maritime Antarctic. *BMC Ecology*, 7, 15. <https://doi.org/10.1186/1472-6785-7-15>
- Bokhorst, S., Huiskes, A. H. L., Convey, P., van Bodegom, P. M., & Aerts, R. (2008). Climate change effects on soil arthropod communities from the Falkland Islands and the Maritime Antarctic. *Soil Biology & Biochemistry*, 40, 1547–1556. <https://doi.org/10.1016/j.soilbio.2008.01.017>
- Bracegirdle, T. J., Colleoni, F., Abram, N. J., Bertler, N. A. N., Dixon, D. A., England, M., Favier, V., Fogwill, C. J., Fyfe, J. C., Goodwin, I., Goosse, H., Hobbs, W., Jones, J. M., Keller, E. D., Khan, A. L., Phipps, S. J., Raphael, M. N., Russell, J., Sime, L., ... Wainer, I. (2019). Back to the future: Using long-term observational and paleo-proxy reconstructions to improve model projections of antarctic climate. *Geosciences*, 9, 255. <https://doi.org/10.3390/geosciences9060255>
- Brooks, M. L. (2003). Effects of increased soil nitrogen on the dominance of alien annual plants in the Mojave Desert. *Journal of Applied Ecology*, 40, 344–353. <https://doi.org/10.1046/j.1365-2664.2003.00789.x>
- Cavieres, L. A., Sanhueza, A. K., Torres-Mellado, G., & Casanova-Katny, A. (2018). Competition between native Antarctic vascular plants and invasive *Poa annua* changes with temperature and soil nitrogen availability. *Biological Invasions*, 20, 1597–1610. <https://doi.org/10.1007/s10530-017-1650-7>
- Cocks, M. P., Newton, I. P., & Stock, W. D. (1998). Bird effects on organic processes in soils from five microhabitats on a nunatak with and without breeding snow petrels in Dronning Maud Land, Antarctica. *Polar Biology*, 20, 112–120. <https://doi.org/10.1007/s003000050284>
- Convey, P., Chown, S. L., Clarke, A., Barnes, D. K. A., Bokhorst, S., Cummings, V., Ducklow, H. W., Frati, F., Green, T. G. A., Gordon, S., Griffiths, H. J., Howard-Williams, C., Huiskes, A. H. L., Laybourn-Parry, J., Lyons, W. B., McMinn, A., Morley, S. A., Peck, L. S., Quesada, A., ... Wall, D. H. (2014). The spatial structure of Antarctic biodiversity. *Ecological Monographs*, 84, 203–244. <https://doi.org/10.1890/12-2216.1>
- Cornelissen, J. H. C., Lang, S. I., Soudzilovskaia, N. A., & During, H. J. (2007). Comparative cryptogam ecology: A review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany*, 99, 987–1001. <https://doi.org/10.1093/aob/mcm030>

- Corte, A. (1961). La primera fanerogama adventicia hallada en el continente Antártico. *Instituto Antártico Argentino*, 62, 1–14.
- Davey, M. C., & Rothery, P. (1996). Seasonal variation in respiratory and photosynthetic parameters in three mosses from the maritime Antarctic. *Annals of Botany*, 78, 719–728. <https://doi.org/10.1006/anbo.1996.0182>
- Dawson, W., Fischer, M., & van Kleunen, M. (2012). Common and rare plant species respond differently to fertilisation and competition, whether they are alien or native. *Ecology Letters*, 15, 873–880. <https://doi.org/10.1111/j.1461-0248.2012.01811.x>
- Drake, P., Grimshaw-Surette, H., Heim, A., & Lundholm, J. (2018). Mosses inhibit germination of vascular plants on an extensive green roof. *Ecological Engineering*, 117, 111–114. <https://doi.org/10.1016/j.ecoleng.2018.04.002>
- During, H. J., & Tooren, B. F. (1990). Bryophyte interactions with other plants. *Botanical Journal of the Linnean Society*, 104, 79–98. <https://doi.org/10.1111/j.1095-8339.1990.tb02212.x>
- Edwards, J. A. (1979). An experimental introduction of vascular plants from South Georgia to the Maritime Antarctic. *British Antarctic Survey Bulletin*, 49, 73–80.
- Ellis, J. C. (2005). Marine birds on land: A review of plant biomass, species richness, and community composition in seabird colonies. *Plant Ecology*, 181, 227–241. <https://doi.org/10.1007/s11258-005-7147-y>
- Engelen, A., Convey, P., Hodgson, D. A., Worland, M. R., & Ott, S. (2008). Soil properties of an Antarctic inland site: Implications for ecosystem development. *Polar Biology*, 31, 1453–1460. <https://doi.org/10.1007/s00300-008-0486-0>
- Erskine, P. D., Bergstrom, D. M., Schmidt, S., Stewart, G. R., Tweedie, C. E., & Shaw, J. D. (1998). Subantarctic Macquarie Island - A model ecosystem for studying animal-derived nitrogen sources using N-15 natural abundance. *Oecologia*, 117, 187–193. <https://doi.org/10.1007/s004420050647>
- Farji-Brener, A. G., Lescano, N., & Ghermandi, L. (2010). Ecological engineering by a native leaf-cutting ant increases the performance of exotic plant species. *Oecologia*, 163, 163–169. <https://doi.org/10.1007/s00442-010-1589-1>
- Fenton, J. H. C., & Smith, R. I. L. (1982). Distribution, composition and general characteristics of the moss banks of the Maritime Antarctic. *British Antarctic Survey Bulletin*, 51, 215–236.
- Flores-Moreno, H., Reich, P. B., Lind, E. M., Sullivan, L. L., Seabloom, E. W., Yahdjian, L., MacDougall, A. S., Reichmann, L. G., Alberti, J., Báez, S., Bakker, J. D., Cadotte, M. W., Caldeira, M. C., Chaneton, E. J., D'Antonio, C. M., Fay, P. A., Firn, J., Hagenah, N., Harpole, W. S., ... Borer, E. T. (2016). Climate modifies response of non-native and native species richness to nutrient enrichment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20150273. <https://doi.org/10.1098/rstb.2015.0273>
- Frenot, Y., Chown, S. L., Whinam, J., Selkirk, P. M., Convey, P., Skotnicki, M., & Bergstrom, D. M. (2005). Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews*, 80, 45–72. <https://doi.org/10.1017/S1464793104006542>
- Galera, H., Rudak, A., Czyż, E. A., Chwedorzewska, K. J., Znój, A., & Wódkiewicz, M. (2019). The role of the soil seed store in the survival of an invasive population of *Poa annua* at Point Thomas Oasis, King George Island, maritime Antarctica. *Global Ecology and Conservation*, 19, e00679. <https://doi.org/10.1016/j.gecco.2019.e00679>
- Gharajehdaghpoor, T., Roth, J. D., Fafard, P. M., & Markham, J. H. (2016). Arctic foxes as ecosystem engineers: increased soil nutrients lead to increased plant productivity on fox dens. *Scientific Reports*, 6, 24020. <https://doi.org/10.1038/srep24020>
- Gimingham, C. H., & Smith, R. I. L. (1971). Growth form and water relations of mosses in the Maritime Antarctic. *British Antarctic Survey Bulletin*, 25, 1–21.
- Glime, J. M. (2017). Temperature: Effects. In J. M. Glime (Ed.), *Bryophyte Ecology*, Vol. 1 (pp. 1–22). Michigan Technological University and the International Association of Bryologists.
- Greenwood, D. J., Lemaire, G., Gosse, G., Cruz, P., Draycott, A., & Neeteson, J. J. (1990). Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of Botany*, 66, 425–436. <https://doi.org/10.1093/oxfordjournals.aob.a088044>
- Gremmen, N. J. M., Chown, S. L., & Marshall, D. J. (1998). Impact of the introduced grass *Agrostis stolonifera* on vegetation and soil fauna communities at Marion Island, sub-Antarctic. *Biological Conservation*, 85, 223–231. [https://doi.org/10.1016/S0006-3207\(97\)00178-X](https://doi.org/10.1016/S0006-3207(97)00178-X)
- Grömping, U. (2006). Relative Importance for Linear Regression in R: The Package relaimpo. *Journal of Statistical Software*, 17, 1–27.
- He, W.-M., Yu, G.-L., & Sun, Z.-K. (2011). Nitrogen deposition enhances *Bromus tectorum* invasion: Biogeographic differences in growth and competitive ability between China and North America. *Ecography*, 34, 1059–1066. <https://doi.org/10.1111/j.1600-0587.2011.06835.x>
- Hedenäs, L. (2011). Global phylogeography in *Sanionia uncinata* (Amblystegiaceae: Bryophyta). *Botanical Journal of the Linnean Society*, 168, 19–42. <https://doi.org/10.1111/j.1095-8339.2011.01189.x>
- Hilderbrand, G. V., Hanley, T. A., Robbins, C. T., & Schwartz, C. C. (1999). Role of brown bears (*Ursus arctos*) in the flow of marine nitrogen into a terrestrial ecosystem. *Oecologia*, 121, 546–550. <https://doi.org/10.1007/s004420050961>
- Hill, P. W., Broughton, R., Bougoure, J., Havelange, W., Newsham, K. K., Grant, H., Murphy, D. V., Clode, P., Ramayah, S., Marsden, K. A., Quilliam, R. S., Roberts, P., Brown, C., Read, D. J., Deluca, T. H., Bardgett, R. D., Hopkins, D. W., & Jones, D. L. (2019). Angiosperm symbioses with non-mycorrhizal fungal partners enhance N acquisition from ancient organic matter in a warming maritime Antarctic. *Ecology Letters*, 22, 2111–2119. <https://doi.org/10.1111/ele.13399>
- Hrbáček, F., Cannone, N., Kňazková, M., Malfasi, F., Convey, P., & Guglielmin, M. (2020). Effect of climate and moss vegetation on ground surface temperature and the active layer among different biogeographical regions in Antarctica. *Catena*, 190, 104562. <https://doi.org/10.1016/j.catena.2020.104562>
- Hughes, K. A., & Convey, P. (2012). Determining the native/non-native status of newly discovered terrestrial and freshwater species in Antarctica - Current knowledge, methodology and management action. *Journal of Environmental Management*, 93, 52–66. <https://doi.org/10.1016/j.jenvman.2011.08.017>
- Hughes, K. A., Pescott, O. L., Peyton, J., Adriaens, T., Cottier-Cook, E. J., Key, G., Rabitsch, W., Tricarico, E., Barnes, D. K. A., Baxter, N., Belchier, M., Blake, D., Convey, P., Dawson, W., Frohlich, D., Gardiner, L. M., González-Moreno, P., James, R., Malumphy, C., ... Roy, H. E. (2020). Invasive non-native species likely to threaten biodiversity and ecosystems in the Antarctic Peninsula region. *Global Change Biology*, 26, 2702–2716. <https://doi.org/10.1111/gcb.14938>
- IPCC. (2013). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Retrieved from Cambridge.
- Jeschke, M., & Kiehl, K. (2008). Effects of a dense moss layer on germination and establishment of vascular plants in newly created calcareous grasslands. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 203, 557–566. <https://doi.org/10.1016/j.flora.2007.09.006>
- Kołodziejek, J. (2019). Growth performance and emergence of invasive alien *Rumex confertus* in different soil types. *Scientific Reports*, 9, 19678. <https://doi.org/10.1038/s41598-019-56068-9>
- Lindeboom, H. J. (1984). The nitrogen pathway in a penguin rookery. *Ecology*, 65, 269–277. <https://doi.org/10.2307/1939479>
- Line, M. A. (1992). Nitrogen fixation in the sub-Antarctic Macquarie Island. *Polar Biology*, 11, 601–606. <https://doi.org/10.1007/BF00237954>

- Littschwager, J., Lauerer, M., Blagodatskaya, E., & Kuzyakov, Y. (2010). Nitrogen uptake and utilisation as a competition factor between invasive *Duchesnea indica* and native *Fragaria vesca*. *Plant and Soil*, 331, 105–114. <https://doi.org/10.1007/s11104-009-0236-2>
- Liu, G., Yang, Y.-B., & Zhu, Z.-H. (2018). Elevated nitrogen allows the weak invasive plant *Galinsoga quadriradiata* to become more vigorous with respect to inter-specific competition. *Scientific Reports*, 8, 3136. <https://doi.org/10.1038/s41598-018-21546-z>
- Liu, Y., & van Kleunen, M. (2017). Responses of common and rare aliens and natives to nutrient availability and fluctuations. *Journal of Ecology*, 105, 1111–1122. <https://doi.org/10.1111/1365-2745.12733>
- Longton, R. E. (1966). Alien vascular plants on Deception Island, South Shetland Islands. *British Antarctic Survey Bulletin*, 9, 55–60.
- Makarov, M. (2009). The nitrogen isotopic composition in soils and plants: Its use in environmental studies (A Review). *Eurasian Soil Science*, 42, 1335–1347. <https://doi.org/10.1134/S1064229309120035>
- McKendrick, J. D., Batzli, G. O., Everett, K. R., & Swanson, J. C. (1980). Some effects of mammalian herbivores and fertilization on tundra soils and vegetation. *Arctic and Alpine Research*, 12, 565–578. <https://doi.org/10.1080/00040851.1980.12004213>
- Menuez, D. R., & Kettenring, K. M. (2013). The importance of roads, nutrients, and climate for invasive plant establishment in riparian areas in the northwestern United States. *Biological Invasions*, 15, 1601–1612. <https://doi.org/10.1007/s10530-012-0395-6>
- Myers-Smith, I. H., & Hik, D. S. (2013). Shrub canopies influence soil temperatures but not nutrient dynamics: an experimental test of tundra snow–shrub interactions. *Ecology and Evolution*, 3, 3683–3700. <https://doi.org/10.1002/ece3.710>
- Myers-Smith, I. H., Kerby, J. T., Phoenix, G. K., Bjerke, J. W., Epstein, H. E., Assmann, J. J., John, C., Andreu-Hayles, L., Angers-Blondin, S., Beck, P. S. A., Berner, L. T., Bhatt, U. S., Bjorkman, A. D., Blok, D., Bryn, A., Christiansen, C. T., Cornelissen, J. H. C., Cunliffe, A. M., Elmendorf, S. C., ... Wipf, S. (2020). Complexity revealed in the greening of the Arctic. *Nature Climate Change*, 10, 106–117. <https://doi.org/10.1038/s41558-019-0688-1>
- Oishi, Y. (2018). Evaluation of the water-storage capacity of bryophytes along an altitudinal gradient from temperate forests to the alpine zone. *Forests*, 9, 433. <https://doi.org/10.3390/f9070433>
- Olech, M., & Chwedorzewska, K. J. (2011). Short Note: The first appearance and establishment of an alien vascular plant in natural habitats on the forefield of a retreating glacier in Antarctica. *Antarctic Science*, 23, 153–154. <https://doi.org/10.1017/S0954102010000982>
- Otero, X. L., De La Peña-Lastra, S., Pérez-Alberti, A., Ferreira, T. O., & Huerta-Díaz, M. A. (2018). Seabird colonies as important global drivers in the nitrogen and phosphorus cycles. *Nature Communications*, 9, 246. <https://doi.org/10.1038/s41467-017-02446-8>
- Pertierra, L. R., Baker, M., Howard, C., Vega, G. C., Olalla-Tarraga, M. A., & Scott, J. (2016). Assessing the invasive risk of two non-native *Agrostis* species on sub-Antarctic Macquarie Island. *Polar Biology*, 39, 2361–2371. <https://doi.org/10.1007/s00300-016-1912-3>
- Pouliot, R., Rochefort, L., & Gauthier, G. (2009). Moss carpets constrain the fertilizing effects of herbivores on graminoid plants in arctic polygon fens. *Botany-Botanique*, 87, 1209–1222. <https://doi.org/10.1139/B09-069>
- Pyšek, P., Hulme, P. E., Simberloff, D., Bacher, S., Blackburn, T. M., Carlton, J. T., Dawson, W., Essl, F., Foxcroft, L. C., Genovesi, P., Jeschke, J. M., Kühn, I., Liebhold, A. M., Mandrak, N. E., Meyerson, L. A., Pauchard, A., Pergl, J., Roy, H. E., Seebens, H., ... Richardson, D. M. (2020). Scientists' warning on invasive alien species. *Biological Reviews*, 95, 1511–1534. <https://doi.org/10.1111/brv.12627>
- R-Core-Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Ross, K., Ehrenfeld, J., & Patel, M. (2011). The effects of nitrogen addition on the growth of two exotic and two native forest understorey plants. *Biological Invasions*, 13, 2203–2216. <https://doi.org/10.1007/s10530-011-0034-7>
- Serpe, M. D., Orm, J. M., Barks, T., & Rosentreter, R. (2006). Germination and seed water status of four grasses on moss-dominated biological soil crusts from arid lands. *Plant Ecology*, 185, 163–178. <https://doi.org/10.1007/s11258-005-9092-1>
- Shaver, G., & Chapin, F. III (1980). Response to fertilization by various plant growth forms in an Alaskan tundra: Nutrient accumulation and growth. *Ecology*, 61, 662–675. <https://doi.org/10.2307/1937432>
- Smith, R. I. L. (1996). Introduced plants in Antarctica: Potential impacts and conservation issues. *Biological Conservation*, 76, 135–146. [https://doi.org/10.1016/0006-3207\(95\)00099-2](https://doi.org/10.1016/0006-3207(95)00099-2)
- Soudzilovskaia, N. A., Bodegom, P. M., & Cornelissen, J. H. C. (2013). Dominant bryophyte control over high-latitude soil temperature fluctuations predicted by heat transfer traits, field moisture regime and laws of thermal insulation. *Functional Ecology*, 27, 1442–1454. <https://doi.org/10.1111/1365-2435.12127>
- Soudzilovskaia, N. A., Graae, B. J., Douma, J. C., Grau, O., Milbau, A., Shevtsova, A., Wolters, L., & Cornelissen, J. H. C. (2011). How do bryophytes govern generative recruitment of vascular plants? *New Phytologist*, 190, 1019–1031. <https://doi.org/10.1111/j.1469-8137.2011.03644.x>
- Stanton, D. E., Merlin, M., Bryant, G., & Ball, M. C. (2014). Water redistribution determines photosynthetic responses to warming and drying in two polar mosses. *Functional Plant Biology*, 41, 178–186. <https://doi.org/10.1071/fp13160>
- Stoy, P. C., Street, L. E., Johnson, A. V., Prieto-Blanco, A., & Ewing, S. A. (2012). Temperature, heat flux, and reflectance of common subarctic mosses and lichens under field conditions: Might changes to community composition impact climate-relevant surface fluxes? *Arctic, Antarctic, and Alpine Research*, 44, 500–508. <https://doi.org/10.1657/1938-4246-44.4.500>
- Stuiver, B. M., Wardle, D. A., Gundale, M. J., & Nilsson, M. C. (2014). The impact of moss species and biomass on the growth of *Pinus sylvestris* tree seedlings at different precipitation frequencies. *Forests*, 5, 1931–1951. <https://doi.org/10.3390/f5081931>
- Thompson, K., Grime, J. P., & Mason, G. (1977). Seed germination in response to diurnal fluctuations of temperature. *Nature*, 267, 147–149. <https://doi.org/10.1038/267147a0>
- Tooren, B. (1990). Effect of a bryophyte layer on the emergence of seedlings of chalk grassland species. *Acta Oecologica*, 11, 155–163.
- Turner, J., Barrand, N. E., Bracegirdle, T. J., Convey, P., Hodgson, D. A., Jarvis, M., Jenkins, A., Marshall, G., Meredith, M. P., Roscoe, H., Shanklin, J., French, J., Goose, H., Guglielmin, M., Gutt, J., Jacobs, S., Kennicutt, M. C., Masson-Delmotte, V., Mayewski, P., ... Klepikov, A. (2014). Antarctic climate change and the environment: an update. *Polar Record*, 50, 237–259. <https://doi.org/10.1017/S0032247413000296>
- Vasquez, E., Sheley, R., & Svejcar, T. (2008). Nitrogen enhances the competitive ability of cheatgrass (*Bromus tectorum*) relative to native grasses. *Invasive Plant Science and Management*, 1(287–295), 289. <https://doi.org/10.1614/IPSM-08-062.1>
- Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (4th ed.). Springer.
- Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13, 87–115. <https://doi.org/10.1007/BF00002772>
- Wasley, J., Robinson, S. A., Lovelock, C. E., & Popp, M. (2006). Climate change manipulations show Antarctic flora is more strongly affected by elevated nutrients than water. *Global Change Biology*, 12, 1800–1812. <https://doi.org/10.1111/j.1365-2486.2006.01209.x>
- Wasley, J., Robinson, S. A., Turnbull, J. D., King, D. H., Wanek, W., & Popp, M. (2012). Bryophyte species composition over moisture gradients

- in the Windmill Islands, East Antarctica: Development of a baseline for monitoring climate change impacts. *Biodiversity*, 13, 257–264. <https://doi.org/10.1080/14888386.2012.712636>
- Wasowicz, P., Sennikov, A. N., Westergaard, K. B., Spellman, K., Carlson, M., Gillespie, L. J., Saarela, J. M., Seefeldt, S. S., Bennett, B., Bay, C., Ickert-Bond, S., & Väre, H. (2020). Non-native vascular flora of the Arctic: Taxonomic richness, distribution and pathways. *Ambio*, 49, 693–703. <https://doi.org/10.1007/s13280-019-01296-6>
- Zamfir, M. (2000). Effects of bryophytes and lichens on seedling emergence of alvar plants: Evidence from greenhouse experiments. *Oikos*, 88, 603–611. <https://doi.org/10.1034/j.1600-0706.2000.880317.x>
- Zhang, X.-D., Jia, X., Chen, Y.-Y., Shao, J.-J., Wu, X.-R., Shang, L., & Li, B. (2013). Crabs mediate interactions between native and invasive salt marsh plants: A mesocosm study. *PLoS One*, 8, e74095. <https://doi.org/10.1371/journal.pone.0074095>
- Zhu, R., Wang, Q., Ding, W., Wang, C., Hou, L., & Ma, D. (2014). Penguins significantly increased phosphine formation and phosphorus contribution in maritime Antarctic soils. *Scientific Reports*, 4, 7055. <https://doi.org/10.1038/srep07055>
- Zwolicki, A., Zmudczyńska-Skarbek, K., Matuła, J., Wojtuń, B., & Stempniewicz, L. (2016). Differential responses of Arctic vegetation to nutrient enrichment by plankton- and fish-eating colonial seabirds in Spitsbergen. *Frontiers in Plant Science*, 7, 01959. <https://doi.org/10.3389/fpls.2016.01959>

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