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 (δ¹⁵N) 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 δ¹⁵N (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
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; Olishi, 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 (Cornellissen 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 in 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 Andorea, 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–Warnstorffia 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; Gharajedaghipour 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 Andorea 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 δ15N (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...
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° S 45° W) and Anchorage Island (67° S 68° 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) (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 ~3°C in the second half of the 20th century and a warming trend of ~0.5°C/decade has been reported and is predicted for the remainder of the 21st 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° 43’ S, 45° 36’ W) and Byers Peninsula (Livingston Island; 62° 39’ S, 61° 05’ 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 $\delta^{15}$N (-8.21–13.06‰) across the 124 sampled mosses (Table 1). Moss nitrogen and $\delta^{15}$N was quantified on additional moss samples collected close to (<0.5 m) each sampling location for a separate study (Bokhorst et al., 2019). We assumed that the nitrogen and $\delta^{15}$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* (Cruydthoeck.nl;
carpet-forming moss is a turf-forming moss and Andreaea spp. grew for isotope analyses. Values where sufficient grass biomass (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 due to differences in evapotranspiration rates. 2°C and 7°C treatments. Moss-specific effects on temperature and 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.

### 2.1 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 δ¹⁵N 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 δ¹⁵N. 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, δ¹⁵N 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

| Location | Species     | Mean (SE) | 2°C | 7°C | (n) |
|----------|-------------|-----------|-----|-----|-----|
| Nitrogen (%) |             |           |     |     |     |
| Signy Isl. | A. depressinervis | 0.89 (0.10) | 0.93 (0.17) | 0.85 (0.12) | 29  |
| Byers Pen  | A. regularis  | 1.45 (0.13) | 1.48 (0.15) | 1.42 (0.15) | 50  |
| Signy Isl. | C. aciphyllum | 0.70 (0.08) | 0.62 (0.11) | 0.77 (0.15) | 20  |
| Byers Pen  | S. uncinata   | 0.96 (0.13) | 0.92 (0.17) | 1.00 (0.20) | 25  |

| δ¹⁵N (%) |             |           |     |     |     |
|----------|-------------|-----------|-----|-----|-----|
| Signy Isl. | A. depressinervis | −2.72 (0.86) | −1.71 (1.45) | −3.99 (0.49) | 18  |
| Byers Pen  | A. regularis  | 1.73 (0.71) | 1.38 (0.94) | 2.08 (1.09) | 44  |
| Signy Isl. | C. aciphyllum | 1.92 (0.98) | 2.27 (0.88) | 1.83 (1.23) | 10  |
| Byers Pen  | S. uncinata   | 4.60 (1.76) | 7.93 (2.47) | 1.82 (1.99) | 11  |

| Moss depth (mm) |             |           |     |     |     |
|-----------------|-------------|-----------|-----|-----|-----|
| Signy Isl.      | A. depressinervis | 34.8 (1.6) | 33.1 (1.9) | 36.4 (2.6) | 27  |
| Byers Pen       | A. regularis  | 29.5 (0.9) | 28.0 (1.3) | 31.0 (1.1) | 47  |
| Signy Isl.      | C. aciphyllum | 40.9 (4.2) | 37.5 (4.3) | 43.9 (7.9) | 17  |
| Byers Pen       | S. uncinata   | 31.1 (1.4) | 29.6 (1.5) | 33.3 (2.9) | 22  |
case of significant species × 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, δ¹⁵N, 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 δ¹⁵N signature was greatest among both Andreaea species and least for C. aciphyllum. Mean isotopic δ¹⁵N 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×T | TxN |
|---------|-------------|----------|-------|------|
| 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.
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 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 (F1,68 = 22.9, p < 0.001) at 2°C (1.86 ± 0.12‰) compared to 7°C (1.31 ± 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 δ15N 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 δ15N with that of A. depressinervis while no correlations were observed for grass δ15N grown among C. aciphyllum or S. uncinata. The mean grass δ15N signature differed when compared between these three moss species (F2,38 = 4.3, p = 0.020) with the lowest δ15N values found when grown among A. depressinervis (0.35 ± 0.43‰) followed by C. aciphyllum (2.20 ± 0.63‰) and S. uncinata (3.42 ± 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
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 $A. \text{regularis}$, allowing about 75 days for subsequent growth, while this was reduced to 50 days for the other cushion-forming moss $A. \text{depressinervis}$, 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
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 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

| 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. |
| --- |
| **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, *Sania uncinata*.
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 δ15N 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 δ15N 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 δ15N 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 δ15N 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 δ15N 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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