

1 **A maritime Antarctic study on the impact of marine vertebrates on terrestrial micro-**  
2 **arthropods via nutrient content of vegetation**

3 *Trophic cascades in the Antarctic*

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17 **Abstract**

18 Traits of primary producers associated with tissue quality are commonly assumed to play a  
19 strong control on higher trophic levels. However, this view is largely based on studies of  
20 vascular plants, and cryptogamic vegetation has received far less attention. In this study we  
21 utilised natural gradients in nutrient concentrations in cryptogams associated with the  
22 proximity of penguin colonies on a maritime Antarctic island, to quantify the impact of  
23 nitrogen content for micro-arthropod communities.

24 Proximity to penguin colonies increased the nitrogen concentration of cryptogams and the  
25 penguin source was confirmed by decreasing  $\delta^{15}\text{N}$  values at greater distance from colonies.  
26 Micro-arthropod abundance, diversity ( $H'$ ) and richness declined with distance from the  
27 penguin colonies, and was positively correlated with the nitrogen concentrations of  
28 cryptogams.  $\Delta^{15}\text{N}$  of micro-arthropods was positively correlated ( $r^2 = 0.865$ ,  $P < 0.01$ ) with  
29  $\delta^{15}\text{N}$  of the moss *Andreaea depressinervis*, indicating that penguin-derived nitrogen moves  
30 through Antarctic food webs across multiple trophic levels.

31 Nitrogen content of cryptogams was correlated with associated micro-arthropods indicating  
32 that biotic interactions affect community development in Antarctic terrestrial ecosystems. The  
33 spatial patterns of Antarctic biodiversity can therefore be affected by local factors, such as  
34 marine vertebrates, beyond existing latitudinal patterns of temperature and water availability.

35 Keywords: Acari; Collembola; Isotope; Lichen; Moss; Nitrogen

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## 38 **Introduction**

39           Functional traits of vascular plant primary producers can be major drivers of the  
40 community composition of herbivores and decomposers (Grime et al. 1996; Agrawal and  
41 Fishbein 2006; Loranger et al. 2012). Nutrient content of plant tissues, in particular, plays an  
42 important role in supporting the diversity and abundance of consumers (Loranger et al. 2012).  
43 However, patterns described to date are primarily based on studies of vascular plants (Mattson  
44 1980; Grime 2001). Cryptogams (bryophytes, lichens and algae), in contrast have received  
45 less attention despite the large and even dominant role these primary producers play in many  
46 ecosystems, especially at higher latitudes and altitudes, and in the global carbon cycle (Davis  
47 1981; Convey 2013; Convey et al. 2014). The few studies examining cryptogam traits in  
48 relation to consumption have mainly focused on the inhibitory function of secondary  
49 compounds in preventing herbivory (Gauslaa 2005; Asplund and Wardle 2013). The most  
50 extensive study to date on the impacts of physiological traits of lichens for invertebrate  
51 communities found variable responses from different invertebrate groups to lichen nutrient  
52 content which was, in part, due to the variety in lichen growth forms across the different  
53 growth locations that were studied (Bokhorst et al. 2015). To overcome the variability caused  
54 by differences in growth form and growth location, natural gradients in intra-specific lichen  
55 nutrient concentrations are required.

56           Mosses, lichens and algae are the major macroscopic primary producer components of  
57 food webs in many polar terrestrial ecosystems (Convey 2013) and can often be found in the  
58 vicinity of penguin colonies. These penguin colonies and other vertebrate aggregations along  
59 the coast of the Antarctic Peninsula and many offshore islands provide large quantities of  
60 nitrogen that encourage plant growth (Lindeboom 1984; Erskine et al. 1998). Bird-derived  
61 nitrogen sources are well known to support vigorous growth of primary producers globally

62 (Anderson and Polis 1999; Sanchez-Pinero and Polis 2000; Ellis 2005) and to increase  
63 ecosystem process rates (Bokhorst et al. 2007a). The impacts generally diminish with  
64 increasing distance from the source, creating a nutrient gradient within the vegetation which  
65 also includes declines in  $\delta^{15}\text{N}$  (Erskine et al. 1998; Zmudczyńska et al. 2012; Crittenden et al.  
66 2015). Such gradients in  $\delta^{15}\text{N}$  are useful to determine whether higher trophic levels feed on  
67 the penguin-affected vegetation, as  $\delta^{15}\text{N}$  levels typically increase by 2-3 units per step in  
68 trophic level (Peterson and Fry 1987). To date, the existence or magnitude of any impact of  
69 increased nutrient content of primary producers for higher trophic levels in Antarctic  
70 terrestrial ecosystems is poorly understood (Davis 1981).

71 To address these issues we tested whether proximity to penguin colonies was  
72 associated with increased nitrogen concentrations and  $\delta^{15}\text{N}$  values in cryptogams on Signy  
73 Island (maritime Antarctic) and whether this, in turn, correlated with the community  
74 composition of the most prominent elements of the terrestrial fauna, springtails and mites  
75 (Convey 2013). We hypothesised that: 1) the nitrogen concentrations and  $\delta^{15}\text{N}$  values of  
76 cryptogams would decrease with increasing distance from penguin colonies; and 2) that, as a  
77 consequence, this would be associated with decreased abundance, diversity ( $H'$ ) and species  
78 richness of micro-arthropods living among these cryptogams. Confirmation of these  
79 hypotheses would provide the first direct evidence for trophic cascades in the Antarctic  
80 terrestrial environment, and add support to recent studies (Caruso et al. 2013) challenging the  
81 long-held view that abiotic factors are the driving forces underlying spatial patterns and  
82 processes in the Antarctic terrestrial biota (Convey 1996; Hogg et al. 2006).

83

## 84 **Materials and Methods**

### 85 *Study sites*

86 This study was performed on Signy Island (60°17'S 45°59'W) in the maritime  
87 Antarctic region north-east of the Antarctic Peninsula. The island is part of the South Orkney  
88 Islands archipelago, has an annual temperature of around -2°C and receives about 400 mm yr<sup>-1</sup>  
89 of precipitation of which most falls as snow (Walton 1982; Royles et al. 2013). Up to 50%  
90 of the island's surface is free of snow and ice during summer (December–February), exposing  
91 some of the best-developed and most diverse cryptogam communities in the Antarctic. There  
92 are four areas hosting large penguin colonies along the coastline of Signy Island (Fig. 1). The  
93 largest of these is at North Point, which is inhabited by Adélie (*Pygoscelis adeliae*), chinstrap  
94 (*P. antarctica*) and gentoo penguins (*P. papua*) (total of 13000 breeding pairs). The colony at  
95 Gourlay Peninsula is dominated by Adélie and chinstrap penguins (12900 breeding pairs).  
96 Smaller colonies are situated along the west coast of the island, with the Cummings Cove area  
97 hosting 7000 chinstrap penguin pairs, and Spindrift Rocks 2000 pairs of Adélie penguins.

98

### 99 *Sampling regime*

100 During December 2013 we sampled mosses, algae and lichens as close as possible to  
101 the four penguin colonies and at four additional sites at increasing distances away from each  
102 colony along each of three replicate transects, with sampling points separated by at least 10 m  
103 from each other. Due to the differences in vegetation development and topography between  
104 the four colonies we were unable to collect all cryptogam species from each transect and  
105 sampling distances from the penguin colonies differed (for details see Table 1, Fig. 1). To  
106 determine the impact of cryptogam nitrogen concentrations on micro-arthropod communities  
107 across larger geographical scales we also sampled lichens along the north-south axis of Signy  
108 Island, thereby extending the transect's starting from the North Point penguin colony. In  
109 addition, as some of the transects increased in altitude (up to 200 m asl) at greater distance  
110 from the penguin colonies, we also included three transects along a hillside remote from any

111 penguin colonies in order to control for any potential effect of altitude on micro-arthropod  
112 community composition (See Fig. 1).

113 We focussed on the dominant lichen species, *Usnea antarctica* (DuRietz), and  
114 *Umbilicaria antarctica* (Frey & I.M. Lamb), the moss *Andreaea depressinervis* (Card.) and  
115 the foliose alga *Prasiola crispa* (Lightfoot). Henceforth these species will be referred to by  
116 their genus name alone. Sampling from extensive moss carpets or turves was avoided in order  
117 to avoid artefacts caused by autocorrelation with the presence or extent of moss, which is  
118 known to be a preferred micro-habitat for many micro-arthropods (Davis 1981; Usher and  
119 Booth 1984; Bokhorst et al. 2014).

120 At each sampling site, *Prasiola* was collected with a PVC corer (7 cm diameter)  
121 including the underlying soil (at most 1 cm if any present) and stored in a plastic container.  
122 Moss and lichen clumps were collected by hand and also stored in individual plastic  
123 containers. Mean cryptogam dry mass was: 5.8 g (sd = 2.8) for *Andreaea*, 3.0 g (1.7) for  
124 *Umbilicaria*, 3.7 g (1.6) for *Usnea* and 1.8 g (0.9) for *Prasiola*. All samples were kept in the  
125 shade at ambient temperatures while in the field and at approximately 5°C in the dark when  
126 stored at the station until extraction (within 24 h) in a modified Tullgren extractor for 24 h.  
127 Extracted Collembola and Acari were preserved in 70% ethanol and identified to species  
128 level, except for the smallest Prostigmata which were grouped together. Micro-arthropod  
129 abundance was expressed as the number of individuals per gram moss, algae or lichen. To  
130 obtain dry vegetation mass of each sample all soil and rock (if any) were removed and the  
131 remaining vegetation weighed when dry after completion of the micro-arthropod extractions.  
132 Moisture content of the sample was quantified by weighing each sample before and after  
133 placement in the Tullgren funnels.

134

135 *Analyses of nitrogen and  $\delta^{15}N$ .*

136 Dried cryptogam samples were transported to Europe for nitrogen and stable isotope  $\delta^{15}\text{N}$   
137 analyses. Nitrogen and  $\delta^{15}\text{N}$  were analysed using a Fisons NA 1500 elemental analyser  
138 coupled to a Finnigan conflo II interface, and a Finnigan MAT Delta S isotope ratio mass  
139 spectrometer. In addition, we measured  $\delta^{15}\text{N}$  values of adult individuals of the springtail  
140 *Cryptogpygus antarcticus* (c. 20 individuals per sample; 210  $\mu\text{g}$ ) and the mites *Alaskozetes*  
141 *antarcticus* and *Halozetes belgicae* (both c. 6 individuals per sample; 280  $\mu\text{g}$ ) to determine if  
142 penguin-derived nitrogen flows from the primary producers to these grazers. The  $\delta^{15}\text{N}$   
143 analyses of micro-arthropods were carried out on samples extracted from *Andreaea* as the  
144 moss contained the highest micro-arthropod abundance. To remove any ethanol before  
145 analyses the micro-arthropods were placed in pre-weighed tin cups and oven dried ( $60^\circ\text{C}$ ) for  
146 24 h. To quantify the biomass of the springtails and mites in each sample the cups were  
147 weighed again after drying before analysis of  $\delta^{15}\text{N}$  (mass ranged from 89-612  $\mu\text{g}/\text{sample}$ ).

148

#### 149 *Calculations and statistical analyses.*

150 To determine differences in nitrogen concentrations,  $\delta^{15}\text{N}$ , water content, abundance  
151 of total micro-arthropods, diversity ( $H'$ ) and species richness along the penguin transects we  
152 used a mixed effect model with distance to the penguin colony as fixed factor and site as a  
153 random factor for each of the cryptogam species separately. To compare the rate of change of  
154 cryptogam traits (%N,  $\delta^{15}\text{N}$  and water content) and the micro-arthropod community along  
155 transects we compared the slopes of linear regression lines through the transect data points  
156 using cryptogam species as fixed factor and site as a random factor. To determine the impact  
157 of penguin colonies on the micro-arthropod variables along greater spatial distances we used  
158 the same mixed effect model as described above but limited to *Usnea* using data from the  
159 North Point and the N-S transects. To account for potential impacts of altitude (as a proxy for  
160 temperature) differences along the transects we compared the differences in *Usnea* N

161 concentrations,  $\delta^{15}\text{N}$ , water content and micro-arthropod variables with altitude along  
162 penguin-influenced transects (North Point, Cummings, and Spindrift Rocks) and non-affected  
163 transects (Moraine Valley) with altitude as a fixed factor and site as random factor.  
164 Correlation coefficients were calculated between the nitrogen,  $\delta^{15}\text{N}$  and water content of  
165 cryptogams and the micro-arthropod variables to explore which if any of these was a stronger  
166 driver behind observed patterns in community composition. Abundance data were log  
167 transformed to achieve homogeneity of variance. All statistical analyses were carried out  
168 using SPSS 22.0 (IBM SPSS Statistics version 22.0. Armonk NY, USA).

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170



171 **Results**

172 *Cryptogam nitrogen and water content*

173 Nitrogen concentrations of cryptogams declined by nearly two-thirds with increasing  
174 distance from penguin colonies (Fig. 2 a-d, Table 2).  $\Delta^{15}\text{N}$  declined by between 5 to 15 units  
175 as the distance to penguin colonies increased, although the variation between species and  
176 different transects was large (Fig. 2e-h). For example, *Prasiola* showed no change in %N or  
177  $\delta^{15}\text{N}$  along the Gourlay transect but it did along the North Point transects (Fig. 2b) while  
178 *Andreaea* showed declines in N along transects of both sites (Fig. 2a). The horizontal extent  
179 of the impact of the penguin colonies on cryptogam N concentrations and  $\delta^{15}\text{N}$  was c. 700-  
180 1000 m for *Andreaea*, 500-750 m for *Prasiola*, 300-500 m for *Usnea* and 700-1500 m for  
181 *Umbilicaria*. Mean nitrogen concentrations and  $\delta^{15}\text{N}$  values were highest ( $F_{3,133} = 64.1$  and  
182 104.7 respectively;  $P < 0.001$ ) for *Prasiola* (4.1%, 10.4‰), followed by *Andreaea* (2.1%,  
183 2.7‰), *Usnea* (1.1%, -2.4‰) and *Umbilicaria* (1.1%, -5.9‰). There were some differences in  
184 water content for individual cryptogams between sites and along transects (Table 2) but not in  
185 a consistent pattern along transects (Fig. S1). Mean water content was highest (ANOVA  $F_{3,133}$   
186 = 30.4  $P < 0.001$ ) for *Prasiola* (67%), followed by *Umbilicaria* (60%), *Andreaea* (52%) and  
187 *Usnea* (25%) (Table 2).

188 *Micro-arthropod communities in relation to distance from penguin colonies, cryptogam*  
189 *nitrogen and water content*

190 There were consistent declines in Acari abundance (3.9 ind./100 m), species richness  
191 (0.2 species/100m) and diversity ( $H'$ ) (0.07/100 m) across cryptogam species and sites (Fig.  
192 3, Table 2) resulting in lower (Tukey HSD  $P < 0.01$ ) values at the furthest transect points  
193 compared to points closest to the penguin colonies. Change in Collembola abundance was

194 variable between species and sites with larger changes along Gourlay compared to North  
195 Point transects among *Prasiola* and *Umbilicaria* resulting in no overall change in Collembola  
196 abundance along transects within the *Prasiola* samples (Fig. 3e). These variable Collembola  
197 patterns were also responsible for the significant species-site interactions for changes in total  
198 micro-arthropods (Table 2).

199         Micro-arthropod communities were positively correlated with nitrogen concentrations  
200 of the cryptogam species but not with that of *Prasiola* (Fig. 4). Acari abundance was  
201 significantly correlated with the nitrogen concentrations of all cryptogam species while  
202 Collembola abundance was significantly correlated with the nitrogen concentrations of  
203 *Andreaea* only (Fig 4a). There were positive correlations between micro-arthropod diversity  
204 ( $H'$ ) and the nitrogen concentrations of the cryptogams (but not that of *Prasiola*), and of  
205 species richness and nitrogen concentrations of the two lichens (Fig. 4).  $\Delta^{15}\text{N}$  for the  
206 collembolan *Cryptopygus antarcticus* was positively correlated with the  $\delta^{15}\text{N}$  values of  
207 *Andreaea* (Fig. 5a), and on average 3.6‰ ( $\pm 0.47$ ) higher than the  $\delta^{15}\text{N}$  of the moss they were  
208 extracted from. Positive correlations were also found for  $\delta^{15}\text{N}$  of the mites *Alaskozetes*  
209 *antarcticus* and *Halozetes belgicae* with that of *Andreaea* (Fig. 5b), and  $\delta^{15}\text{N}$  was 5.8‰ ( $\pm$   
210 0.3) higher compared to the moss they were extracted from. There was no correlation between  
211 micro-arthropods (total abundance, Acari and Collembola) and tissue water content within  
212 cryptogam species. However, when compared across all samples ( $n = 166$ ), total micro-  
213 arthropod and Collembola abundance were positively correlated ( $r^2 = 0.15$  and  $0.24$   
214 respectively  $P < 0.05$ ) with cryptogam water content, reflecting the potential impact of the  
215 different cryptogam growth forms and water holding capacity.

216 *Micro-arthropod communities across Signy Island and in relation to altitude*

217           There were no differences in micro-arthropod abundance, diversity ( $H'$ ) or species  
218 richness along the N-S transect across the island (Fig. 6). Nitrogen concentrations and  $\delta^{15}N$   
219 did not differ along the N-S transect and the penguin impact on *Usnea* N concentrations was  
220 limited to c. 300-500 m distance (Fig. 6c). There were no correlations between *Usnea* N  
221 concentrations and the micro-arthropod community along the N-S transect (data not shown).  
222 The penguin colony impact on micro-arthropod abundance, diversity ( $H'$ ) and species  
223 richness in *Usnea* appears limited to c. 300-500 m distance as all more distant sampling sites  
224 showed no further differences (Fig. 6a,b). There was no difference in the micro-arthropod  
225 abundance or species richness across transects from Moraine Valley to Green Gable (Fig.  
226 6d,e) indicating that there was no effect of altitude on the micro-arthropod community along  
227 the study transects.  $\delta^{15}N$  of *Usnea* did not change with altitude along the Moraine Valley  
228 transects (Fig. 6f) and there was no correlation with the micro-arthropod abundance or species  
229 richness. In comparison with the penguin colony-associated transects the micro-arthropod  
230 abundance, diversity ( $H'$ ) and richness were consistently lower (Tukey HSD  $P < 0.05$ ) along  
231 the Moraine Valley transect (Table 2, Fig. 6).

#### 232 *Micro-arthropod communities across cryptogam species*

233           Species richness was highest (Tukey HSD  $P < 0.01$ ) in *Andreaea* (4 species on  
234 average) compared to the other cryptogams (2 to 3 species) (Table 2). The lowest micro-  
235 arthropod diversity ( $H'$ ) was found among *Prasiola* (mean  $H'$  of 0.3 across all samples) while  
236 the other cryptogam species had higher (Tukey HSD  $P < 0.05$ ) mean values ranging from 0.5  
237 to 0.7. In contrast, total micro-arthropod abundance was highest (Tukey HSD  $P < 0.05$ ) in  
238 *Prasiola* followed by *Andreaea*, *Umbilicaria* and *Usnea* (data not shown). Acari abundance  
239 did not differ between cryptogam species. Collembola abundance was consistently higher

240 (Tukey HSD  $P < 0.001$ ) in *Prasiola* compared to the other cryptogam species, and *Andreaea*  
241 supported more Collembola than the two lichen species.

242 The predatory mite *Gamasellus racovitzai* was found across all cryptogams but  
243 abundances were higher in *Andreaea* and *Prasiola* (data not shown). Abundance of the  
244 predatory mite *G. racovitzai* was positively correlated with Collembola (prey) abundance  
245 across all ( $n = 126$ ) cryptogam samples along transects ( $r^2 = 0.113$ ,  $P < 0.05$ ) but this  
246 correlation improved ( $r^2 = 0.585$ ,  $P < 0.01$ ) when only the *Usnea* data ( $n = 42$ ) were used, as  
247 no significant correlations were present among the other cryptogams individually (data not  
248 shown).

249

250

## 251 **Discussion**

252 This is one of the first studies to examine the role of cryptogam nutrient traits in  
253 relation to micro-arthropod communities, and the first to do this in the Antarctic. Our data  
254 provide clear support for the role of primary producers, as distinct from abiotic interactions,  
255 as a factor contributing to ecosystem development in terrestrial ecosystems of the maritime  
256 Antarctic region (Hogg et al. 2006; Caruso et al. 2013). Although the spatial distribution of  
257 Antarctic terrestrial biodiversity is heavily influenced by temperature and water availability  
258 (Convey et al. 2014), marine vertebrates clearly have a strong impact at local scales (Erskine  
259 et al. 1998; Ellis 2005). Our findings are consistent with general patterns observed for  
260 vascular plants in more temperate regions (Loranger et al. 2012) and therefore extend general  
261 understanding of the drivers of invertebrate abundance and diversity through traits of other  
262 important primary producers such as cryptogams (Bokhorst et al. 2015).

263 Hypothesis 1 was supported by the decrease in nitrogen concentrations and  $\delta^{15}\text{N}$  of  
264 cryptogams with increasing distance from penguin colonies. These findings are consistent  
265 with other studies from polar regions, where plant productivity increased closer to penguin  
266 colonies (Lindeboom 1984) and the stable isotope nitrogen signature of the primary producers  
267 was directly linked to the trophic level of the animals producing the faecal matter (Erskine et  
268 al. 1998; Crittenden et al. 2015). Like many plants in Arctic and temperate regions, Antarctic  
269 primary producers also tend to be nitrogen-limited in their growth (Davey and Rothery 1992;  
270 Wasley et al. 2006) and penguin colonies greatly increase the availability of nitrogen for  
271 cryptogams. The direct impact of penguin colonies here on the nitrogen content (% N and  
272  $\delta^{15}\text{N}$ ) of cryptogams varied greatly between species and sites, ranging from 300-1500 m  
273 distance, which is within the range of observations from Marion Island (Erskine et al. 1998),

274 although Crittenden et al. (2015) reported detectable impacts of penguin-derived ammonia on  
275 lichen physiological activity at distances of up to 10 km.

276         The variation in N concentrations between species and sites with distance to penguin  
277 colonies was most likely caused by site specific factors. For instance, N concentrations of  
278 *Andreaea* and *Prasiola* both declined along North Point transects but along the Gourlay  
279 transects this pattern was only found for *Andreaea*. This lack of response by *Prasiola* to the  
280 obvious N gradients that exist along the Gourlay transects, as shown by N concentration in  
281 *Andreaea* and *Umbilicaria*, may be caused by the decaying moss bank that underlies these  
282 transects (Smith 1988) from which *Prasiola* may receive additional N, while *Andreaea* and  
283 *Umbilicaria* were sampled from rock and therefore not so open to influence by N sources  
284 from below. However, there is no good explanation available for the differences in N content  
285 changes between *Usnea* and *Umbilicaria* along the Cummings Cove transects. Despite Signy  
286 Island being a relatively small island and nutrient inputs from sea spray and marine  
287 vertebrates generally being assumed to reach across the island (Allen et al. 1967) there were  
288 clear changes and limits (c. 300-1500 m horizontal distance) detected here to the impact of  
289 penguin colonies on the nitrogen concentrations or  $\delta^{15}\text{N}$  of cryptogams. Additional factors  
290 that may have affected cryptogam  $\delta^{15}\text{N}$  values include the penguin population size, dominant  
291 penguin species within the colony and overall wind direction that carries the nitrogen towards  
292 the vegetation. A larger comparative study than that presented here would be required to take  
293 these factors into account.

294         The decreases in micro-arthropod abundance, diversity ( $H'$ ) and species richness as  
295 distance to the penguin colonies increased provide support for Hypothesis 2. The micro-  
296 arthropod community values also showed strong correlations with cryptogam nitrogen  
297 concentrations indicating that this may be a driving force for community assembly at higher

298 trophic levels in these Antarctic ecosystems. Water availability and temperature are typically  
299 considered as strong drivers of biological patterns in the Antarctic (Kennedy 1993; Convey et  
300 al. 2014), but we did not observe any effect of intra-specific cryptogam water content or site  
301 temperature (as altitude proxy) on the micro-arthropod community along the sampling  
302 transects. Although site temperature was only studied for *Usnea*, the Gourlay transects  
303 (including other cryptogams) showed strong declines in Acari abundance while only reaching  
304 a third of the altitude of other transects, indicating that altitude/temperature differences along  
305 these transects play a minor role for the studied species. The current data indicate that water  
306 and temperature are of less importance than nutrient availability at closer proximity (c. 500  
307 m) to penguin colonies for Antarctic micro-arthropods at this maritime Antarctic island. This  
308 suggests that, at relatively small geographical scales, different drivers of community assembly  
309 exist in maritime Antarctic terrestrial ecosystems. The existence of such smaller-scale  
310 gradients in driving forces behind community development allows for further detailed  
311 unravelling of these factors by manipulations of nutrient availability (Wasley et al. 2006) and  
312 micro-climate (Bokhorst et al. 2011). Further work should ideally also include other  
313 physiological variables such as phosphorus and defence compounds (Bokhorst et al. 2015;  
314 Crittenden et al. 2015).

315         The strong correlation between the  $\delta^{15}\text{N}$  of the micro-arthropods and that of the moss  
316 *Andreaea* (Fig. 5) indicates that the penguin-derived nitrogen is taken up by the moss and  
317 transferred to the micro-arthropods. The mean increase from moss to springtails was 3.6‰  
318 and is consistent with changes generally associated between trophic levels (Peterson and Fry  
319 1987). However, with the current data it is not possible to determine if the springtails feed on  
320 the live moss, the epiphytic micro-flora living among the moss shoots, or on decaying moss  
321 shoots. The 5.8‰ increase found for the mites compared to the moss (Fig. 5b) is higher than a

322 single trophic level step, suggesting that the mites also feed from other food sources with  
323 higher  $\delta^{15}\text{N}$  values such as penguin faecal matter and dead animals (Goddard 1982; Schneider  
324 et al. 2004). Despite these uncertainties the  $\delta^{15}\text{N}$  values showed a clear pattern along transects  
325 away from penguin colonies in both primary producers and consumers, with consistent  
326 declines in nitrogen concentrations and abundance, diversity and species richness of micro-  
327 arthropods. These results provide strong support for the existence of biotic interactions  
328 between primary producers and higher trophic levels in Antarctic terrestrial ecosystems.

329         The abundance of micro-arthropods, notably Collembola, was not always related to  
330 the nitrogen concentrations of the primary producers and, particularly, the *Prasiola*  
331 communities showed very poor correlations despite the large range in nitrogen concentrations  
332 measured (N: 1.6% - 6.2%). This indicates that other characteristics of cryptogams, such as  
333 phosphorus, defence compounds against herbivory and growth form affecting the micro-  
334 habitat conditions, may also have played a role (Asplund and Wardle 2013; Bokhorst et al.  
335 2015). Overall, the highest micro-arthropod abundance and diversity were found among  
336 *Andreaea* and *Prasiola* compared to the lichens. These differences most likely reflect the  
337 differences in nitrogen concentrations, growth form and water content (there were significant  
338 correlations with water content when compared across all sampled cryptogams, as opposed to  
339 the low variation within species). The compact growth of moss shoots provides a more stable  
340 micro-environment than the open structure of the lichen thallus, and moss has been shown to  
341 have a strong positive effect on the abundance of micro-arthropods in other parts of the world  
342 (Salmane and Brumelis 2008; Bokhorst et al. 2014). The collembolan *Cryptopygus*  
343 *antarcticus* is known to have poor control over its body water content (Block and Harrisson  
344 1995; Convey et al. 2003) and, therefore, would be less prone to desiccation when within the  
345 moss profile. Similarly, the foliose alga *Prasiola*, whose 3D structure is typified by much



346 folding and many small protected cavities, provides a continuous moist environment optimal  
347 for these micro-arthropods. The high abundance of micro-arthropods among *Prasiola*  
348 compared to the cryptogams was most likely driven by the preference for algae as a food  
349 source by at least some of these micro-arthropods (Worland and Lukešová 2000; Bokhorst et  
350 al. 2007b) and the availability of optimal moisture conditions. The main driving forces  
351 underlying the distribution of consumers within Antarctic terrestrial ecosystems may therefore  
352 depend on the specific requirements for growth and avoidance of adverse abiotic conditions  
353 of individual species of micro-arthropod. Further work comparing a larger range of  
354 cryptogam growth forms than used in this study would be required to determine the role of  
355 these for the invertebrate communities in the Antarctic.

356         The current findings indicate a strong link between the nitrogen concentrations of  
357 Antarctic primary producers such as mosses and lichens and the community composition of  
358 associated micro-arthropods. These data extend the general understanding of the influence of  
359 nutrient concentrations in cryptogams on community assembly of invertebrates. Although the  
360 data indicate that the impact of marine vertebrates on cryptogam nutrients and micro-  
361 arthropod community development is important, it also declines at relatively short distances  
362 (c. 300-1500 m) from the source. Micro-arthropod biodiversity patterns in the maritime  
363 Antarctic are driven by availability of water and temperature when considered across large  
364 geographical scales, but biotic interactions can clearly become more important at the local  
365 scale.

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476 **Figures**

477 Figure 1. Map of Signy Island indicating the major penguin colonies. The lines represent the  
478 sampling transects along which samples of cryptogams and micro-arthropods were collected.  
479 The dashed line represents transects without penguin presence from the bottom of Moraine  
480 Valley to the top of Green Gable.

481 Figure 2. Nitrogen concentrations (%) and  $\delta^{15}\text{N}$  values of *Andreaea depressinervis* (a),  
482 *Prasiola crispa* (b), *Usnea antarctica* (c) and *Umbilicaria antarctica* (d) along transects away  
483 from penguin colonies on Signy Island. Note that not all species were present along each  
484 transect. Different letters indicate significant differences (Tukey HSD  $P < 0.05$ ) in N or  $\delta^{15}\text{N}$   
485 values between sampling sites. For clarity these differences are only presented for one  
486 transect but the other transects show similar patterns. Data points are the mean of  $n = 3$  with  
487 SE as error bars. ●: North Point, ○: Gourlay, ▼: Cummings Cove, ■: Spindrift rocks.

488 Figure 3. Micro-arthropod abundance, diversity ( $H'$ ) and species richness along transects  
489 away from penguin colonies on Signy Island. Regression lines are drawn through the mean of  
490 the presented data points. Significant  $r^2$  are indicated by: \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P <$   
491  $0.001$ . Data points are the mean of  $n = 3$  with error bars as SE. ●: North Point, ○: Gourlay, ▼:  
492 Cummings Cove, ■: Spindrift rocks.

493 Figure 4. Regressions between micro-arthropod abundance, diversity ( $H'$ ), richness with the  
494 nitrogen (%) concentration of *Andreaea depressinervis*, *Prasiola crispa*, *Usnea antarctica*  
495 and *Umbilicaria antarctica*. Data points are individual moss, alga and lichen samples  
496 collected along transects away from penguin colonies. Significant  $r^2$  are indicated by: \*  $P <$   
497  $0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ .

498 Figure 5. Regressions between  $\delta^{15}\text{N}$  of *Andreaea depressinervis* and  $\delta^{15}\text{N}$  of micro-  
499 arthropods. a)  $\delta^{15}\text{N}$  of *Cryptopygus antarcticus* from the North Point and Gourlay transects.  
500 The upper dashed regression line through the springtail data points was 3.5‰ higher ( $Y =$   
501  $1.028 X + 3.54$ ) than that of the moss ( $Y = X$ ). b)  $\delta^{15}\text{N}$  of *Alaskozetes antarcticus* and  
502 *Halozetes belgicae* from the Gourlay transects. The upper dashed regression line through the  
503 mite data points was 5.8‰ higher ( $Y = 0.974 X + 6.0$ ) than that of the moss ( $Y = X$ ).  
504 Significant  $r^2$  are indicated by: \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$ .

505 Figure 6. Micro-arthropod abundance and richness in *Usnea antarctica* across Signy Island  
506 and in relation to altitude. a) Micro-arthropod abundance, b) species richness, c) Nitrogen  
507 (%N) and  $\delta^{15}\text{N}$  of *Usnea* along the North Point transects away from the penguin colony (grey  
508 shaded area) and the north-south transect across Signy Island. Data points with different  
509 letters are significantly different (Tukey HSD  $P < 0.05$ ). Note that, for clarity, in c) only the  
510 Tukey differences for  $\delta^{15}\text{N}$  are shown but the %N follows the same pattern. d) Micro-  
511 arthropod abundance, e) species richness, f)  $\delta^{15}\text{N}$  of *Usnea* along the slopes of the penguin-  
512 affected North Point transects and that of Moraine Valley (without penguins). %N of *Usnea*  
513 shows the same pattern as for  $\delta^{15}\text{N}$  but is not shown. Different letters indicate significant  
514 differences (Tukey HSD  $P < 0.05$ ) for each site separately. ANOVA statistics are presented in  
515 Table 2. Data points are the mean of  $n = 3$  with SE as error bars.

516



517 Table 1. Details of cryptogam sampling transects in relation to penguin colonies across Signy Island. For the exact location of each penguin  
 518 colony and the locations of the N-S transect and Moraine Valley see Figure 1. Distances from penguin colonies for the N-S transect were  
 519 measured from North Point for Robin Peak, Springtail Spur and Jane Col, while Snow Hills and Garnet Hill were measured from the Gourlay  
 520 Peninsula. -: not present at site.

Sampling sites	North Point	Gourlay	Cummings	Spindrift rocks	N-S transect	Moraine Valley
	Distance from penguin colony (m) and altitude (m asl.)					altitude (m asl.)
Penguin species	<i>P. adeliae</i> , <i>P. antarctica</i> , <i>P. papua</i>	<i>P. adeliae</i> , <i>P. antarctica</i>	<i>P. antarctica</i>	<i>P. adeliae</i>		
Breeding pairs (n)	13000	12900	7000	2000		
1	0 (30)	0 (10)	20 (20)	150 (20)	Robin Peak	1000 (220) 40
2	300 (50)	150 (20)	100 (50)	200 (40)	Springtail Spur	1700 (160) 60
3	500 (100)	300 (20)	300 (80)	300 (60)	Jane Col	2700 (160) 90
4	700 (150)	700 (60)	600 (150)	500 (100)	Snow Hills	3600 (265) 120
5	800 (200)	1300 (70)	-	700 (150)	Garnet Hill	2100 (227) 206
Species sampled						
<i>Andreaea</i>	+	+				
<i>Prasiola</i>	+	+				
<i>Usnea</i>	+		+	+	+	+
<i>Umbilicaria</i>	+	+	+			

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523 Table 2. Mixed effects ANOVA results of cryptogam differences in N content,  $\delta^{15}\text{N}$ , water content and micro-arthropods in relation to distance  
524 to penguin colonies. The comparison of change in arthropods along transects compares the slopes of linear regression through the data points (as  
525 shown in Fig. 3). The North-South transect statistics show the comparison of the micro-arthropod community among *Usnea* from the North  
526 Point penguin colony to the southern tip of the island (see Table 1). The altitude statistics represent a comparison of the micro-arthropod  
527 community among *Usnea* along three penguin-associated transects (see Table 1) to that of non-penguin affected transects (Moraine Valley).  
528 There were three replicate transects per site and site was used as a random factor in the analyses. Significant differences are indicated by \*  $P <$   
529 0.05, \*\*  $P <$  0.01, \*\*\*  $P <$  0.001.

		Cryptogam			Micro-arthropod abundance			Species richness	Diversity
		%N	$\delta^{15}\text{N}$	Water content	Total	Acari	Collembola	R	(H')
<i>Andreaea</i>	Distance (8,20)	12.2 ***	5.7 ***	1.9	0.1	5.7 **	2.4	6.2 ***	7.8 ***
	Site (1,20)	14.4 ***	0.3	3.9	0.5	0.4	1.6	2.5	0.0
<i>Prasiola</i>	Distance (7,18)	2.0	3.1 *	2.3	2.2	2.4	2.6	2.8 *	4.3 **
	Site (1,18)	0.2	5.5 *	6.9 *	0.1	0.1	6.5 *	0.3	7.5 *
<i>Usnea</i>	Distance (9,30)	5.5 ***	8.4 ***	7.3 ***	13.1 ***	10.3 ***	10.9 ***	6.5 ***	3.1 **
	Site (2,30)	2.4	6.3 **	12.0 ***	2.6	2.5	1.7	2.0	0.3
<i>Umbilicaria</i>	Distance (10,29)	5.2 ***	1.1	3.9 **	1.5	2.3*	1.3	3.7 **	4.5 **
	Site (2,29)	9.3 **	5.6 **	36.5 ***	0.7	1.6	8.8 **	0.3	1.1
Rate of change in micro-arthropods along transects									
	Species (3,20)	1.0	1.1	10.8 *	0.7	1.0	0.2	9.0	0.8
	Site (3,20)	2.4	0.7	2.6	0.3	1.5	0.5	1.7	0.3
	Site×Species (3,20)	9.7 ***	8.9 ***	1.4	9.3 ***	1.9	10.0 ***	0.2	1.3
N-S transect across Signy Island									
<i>Usnea</i>	Distance (8,20)	4.9 **	4.5 **	3.4 *	9.6 ***	4.4 **	18.2 ***	2.1	0.7
	Site (1,20)	0.0	1.6	4.8 *	0.1	0.2	0.7	0.1	0.6
Altitude comparison									
<i>Usnea</i>	Altitude (12,41)	6.3 ***	7.6 ***	2.7 **	8.6 ***	8.3 ***	7.3 ***	6.0 ***	2.2 *
	Site (2,41)	2.4	11.0 ***	20.1 ***	2.4	4.1 *	2.6	0.1	1.3

