PRIFYSGOL BANGOR / BANGOR UNIVERSITY

PRIFYSGOL BANGOR UNIVERSITY

Angiosperm symbioses with non-mycorrhizal fungal partners enhance N acquisition from ancient organic matter in a warming maritime Antarctic

Hill, Paul; Broughton, Richard; Bougoure, Jeremy; Havelange, William; Newsham, Kevin K.; Gran, Helen; Murphy, Daniel V.; Clode, Peta; Ramayah, Soshila; Marsden, Karina; Quilliam, Richard; Roberts, Paula; Brown, Caley; Read, David J.; DeLuca, Thomas; Bardgett, Richard D.; Hopkins, David W.; Jones, Davey L.

Ecology Letters

DOI:
10.1111/ele.13399

Published: 01/12/2019

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Hill, P., Broughton, R., Bougoure, J., Havelange, W., Newsham, K. K., Gran, H., Murphy, D. V., Clode, P., Ramayah, S., Marsden, K., Quilliam, R., Roberts, P., Brown, C., Read, D. J., DeLuca, T., 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*(12), 2111-2119. https://doi.org/10.1111/ele.13399

Hawliau Cyffredinol / General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal ?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

09. Oct. 2020

1 **Angiosperm symbioses with non-mycorrhizal fungal partners enhance N**
2 **acquisition from ancient organic matter in a warming maritime Antarctic**

3

4 Paul W Hill[1], Richard Broughton[2,3], Jeremy Bougoure[4,5], William Havelange[1], Kevin K
5 Newsham[2], Helen Grant[6], Daniel V Murphy[4], Peta Clode[5,7], Soshila Ramayah[1], Karina A
6 Marsden[1,8], Richard S. Quilliam[1,9], Paula Roberts[1], Caley Brown[1], David J Read[10], Thomas H
7 Deluca[1,11], Richard D Bardgett[12], David W Hopkins[13], Davey L Jones[1,4]

8

9 1 School of Natural Sciences, Bangor University, Bangor LL57 2UW, UK.
10 2 British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley
11 Road, Cambridge, CB3 OET, UK.
12 3 Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK
13 4 SoilsWest, UWA School of Agriculture and Environment, Faculty of Science, University of
14 Western Australia, WA 6009, Australia.
15 5 Centre for Microscopy, Characterisation and Analysis, University of Western Australia, WA
16 6009, Australia.
17 6 Life Sciences Mass Spectrometry Facility, Lancaster Environment Centre, Lancaster LA1
18 4AP UK.
19 7 UWA School of Biological Sciences, University of Western Australia, WA 6009, Australia.
20 8 Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville,
21 Victoria 3010, Australia.
22 9 Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling,
23 Stirling, FK9 4LA, UK.
24 10 Animal and Plant Sciences, University of Sheffield, Western bank, Sheffield, S10 2TN, UK
25 11 WA Franke College of Forestry and Conservation, University of Montana, Missoula, MT
26 59812 USA.

27 12 School of Earth and Environmental Sciences, University of Manchester, Manchester, M13
28 9PL, UK.
29 13 SRUC - Scotland's Rural College, West Mains Road, Edinburgh, EH9 3JG, UK.

30

31

32 Running title: DSEs enhance Antarctic plant N acquisition

33 Keywords: Soil; Nitrogen cycle; Carbon cycle; Climate change; Enantiomers; Polar; Dark
34 septate endophytes

35 Type or article: Letter

36 Words in Abstract: 150

37 Words in Main text: 3676

38 Number of references: 40

39 Number of figures: 5

40 Corresponding author:

41 Paul Hill, Environment Centre Wales, School of Natural Sciences, Bangor University, Deiniol
42 Road, Bangor LL57 2UW, UK.

43 Email: p.w.hill@bangor.ac.uk

44 Tel: +44 1248 2632

45 Author Contributions:

46 PH, DJ, KKN, RDB, DH, PR, TD and RQ conceived the investigation; PH carried out
47 fieldwork; RB carried out amino acid analysis; JB, DM and PC carried out nanoSIMS work;
48 PH, WH, CB, SR and KM carried out laboratory experiments and analysis; HG carried out
49 IRMS analysis; PH wrote the manuscript first draft; all authors contributed to the final version.

50 Data accessibility:

51 Data will be archived at Figshare DOI: 10.6084/m9.figshare.9791693 following acceptance.

52

53 **Abstract**

54 In contrast to the situation in plants inhabiting most of the world's ecosystems, mycorrhizal
55 fungi are usually absent from roots of the only two native vascular plant species of maritime
56 Antarctica, *Deschampsia antarctica* and *Colobanthus quitensis*. Instead, a range of ascomycete
57 fungi, termed dark septate endophytes (DSEs), frequently colonise the roots of these plant
58 species. We demonstrate that colonisation of Antarctic vascular plants by DSEs facilitates not
59 only the acquisition of organic nitrogen as early protein breakdown products, but also as non-
60 proteinaceous D-amino acids and their short peptides, accumulated in slowly-decomposing
61 organic matter, such as moss peat. Our findings suggest that, in a warming maritime Antarctic,
62 this symbiosis has a key role in accelerating the replacement of formerly dominant moss
63 communities by vascular plants, and in increasing the rate at which ancient carbon stores laid
64 down as moss peat over centuries or millennia are returned to the atmosphere as CO_2.

65

66 **Introduction**

67 Fungal root symbionts have been crucial to the success of plants in terrestrial ecosystems, with
68 a relationship dating back to the colonisation of land (Strullu-Derrien et al. 2018). Mutualistic
69 relationships with mycorrhizal fungi remain key to the acquisition of limiting nutrients, such
70 as nitrogen (N) and phosphorus (P), in the majority of terrestrial plants (Smith & Read 2008).
71 However, in marked contrast to their presence in most ecosystems, mycorrhizas are typically
72 absent from the roots of vascular plants in maritime Antarctica (Upson et al. 2008; Newsham
73 et al. 2009). In this region, the roots of the two native angiosperms, *Deschampsia antarctica*
74 Desv. (a grass) and *Colobanthus quitensis* (Kunth) Bartl. (a cushion-forming plant, Fig. 1) are
75 instead colonised by a range of ascomycete fungi, collectively termed dark septate endophytes
76 (DSEs) (Fig. 1, Upson et al. 2008; Newsham et al. 2009), which may have a role in the
77 acquisition of organic N from soils (Upson et al. 2009; Newsham 2011).

78 In areas of the maritime Antarctic not under permanent ice, moss cover can be extensive (Fig.
79 S1 in Supporting Information) and dominates primary productivity. It is estimated to account
80 for 45 km^2 of Antarctic Peninsula land area and is particularly prevalent on islands such as the
81 South Orkney and South Shetland Islands (Fretwell et al. 2011; Royles & Griffiths 2015). Due
82 to the constraints imposed on decomposition by low temperatures, moss growth leads to the
83 accumulation of large amounts of soil organic matter, including substantial stores of protein
84 (Royles & Griffiths 2015). Vascular plants, and particularly pioneer individuals and
85 populations, are commonly found amongst mosses, exploiting stored proteinaceous N to
86 facilitate establishment (Fig. 1, Hill et al. 2011a).
87 In a survey of roots of *D. antarctica* and *C. quitensis* on Signy Island (60° 43' S, 45° 36' W)
88 in the South Orkney Islands, maritime Antarctica, we found the most consistent and extensive
89 occurrence of DSE hyphae and characteristic microsclerotia (Fig. 1) was in the roots of plants
90 growing amongst banks formed by the moss *Chorisodontium aciphyllum* (Hook. f. & Wilson)
91 Broth. (Fig. 1). Banks formed by this moss frequently exceed 1 m in depth and may be up to 3
92 m deep, storing organic matter that has remained undecomposed over millennia (Royles et al.
93 2012; Royles & Griffiths 2015, Fig. S2 in Supporting Information). This organic matter has
94 become increasingly bioavailable as mean air temperatures have risen in the maritime
95 Antarctic, leading to progressive thawing of the moss banks (Royles et al. 2012; Abrams et al.
96 2013; Royles & Griffiths 2015; Amesbury et al. 2017).
97 In most cases (e.g., amongst the moss *Sanionia uncinata* (Hedw.) Loeske, Figs. 1 and S1), *D.*
98 *antarctica* appears to root no deeper than *c*. 10 cm, with its roots usually extending to a depth
99 of 5 cm or less (Fig. S3 in Supporting Information), corresponding to the depth of accumulated
100 organic matter. However, in *C. aciphyllum* banks, the grass was observed rooting down to >
101 25 cm, where organic matter may have been stored for > 500 years (Royles et al., 2012). We
102 hypothesised that the penetration of roots colonised by DSEs deep into moss banks allows *D.*

103 *antarctica* to exploit ancient nutrients that up until recent decades were unavailable because
104 the moss banks have been frozen.
105 Due to slow N mineralisation, it is likely that early breakdown products of accumulated
106 proteins (L-amino acids and their short peptides) make a substantial contribution to plant N
107 nutrition in polar soils (Chapin et al. 1993; Hill et al. 2011a). However, peptides containing D-
108 glutamic acid and especially D-alanine are common constituents of bacterial peptidoglycan and
109 various D-amino acids occur in bacteria, archaea, fungi, plants and animals (Yoshimura &
110 Esaki 2003; Friedman 2010; Vranova et al. 2012). D-amino acids are also known to accumulate
111 from proteinaceous L-amino acids during long periods of storage, due to abiotic racemisation,
112 which may take place at a rates of about 0.3% of L-amino acids per decade (Wichern et al.
113 2004). Consequently, D-amino acids accumulate in soils where decomposition is slow e.g., in
114 deserts or in peat, such as that formed by moss banks (Kunnas & Jauhiainen 1993; Wichern et
115 al. 2004).
116 It is clear from previous investigations that both plants and soil microbes are able to take up
117 and metabolise some D-amino acids such as D-alanine (Hill et al. 2011b,c; Hill et al. 2012;
118 Vranova et al. 2012). However, in contrast to short L-peptides, which appear to be widely
119 metabolised, until now, evidence suggested that short D-peptides could be metabolised by soil
120 microbes but not by plants (Hill et al. 2011b,c; Hill et al. 2012; Vranova et al. 2012). Whether
121 the ability to metabolise D-peptides is present in plants inhabiting soils where D-enantiomers
122 are a more available source of N is unknown. We measured uptake of a range of N forms under
123 field conditions in the Antarctic and found that both native vascular plants could acquire N
124 from D-alanine and its dipeptide - as well as from longer peptides of the L-enantiomer than
125 previously recognised. Further, we found that colonisation with DSEs facilitated plant
126 acquisition of N from both L- and D-enantiomers of alanine and their peptides.
127

128 **Materials and Methods**

129 **Assessment of fungal endophyte colonisation**

130 Roots of *D. antarctica* and *C. quitensis* were collected from locations around Signy Island
131 (Gourlay Peninsula; Polynesia Point; Factory Cove; Berntsen Point; Lower slopes of Factory
132 Bluffs; Starfish Cove; North Point; Moss Braes; Deschampsia Point; Foca Cove; Fig. S4 in
133 Supporting Information). Roots were washed in water and examined for the presence of DSE
134 hyphae and microsclerotia by light microscopy after staining (Newsham & Bridge 2010). The
135 same analyses confirmed the absence of arbuscular mycorrhizal structures from roots (Upson
136 et al. 2008).

137 **Soil solution collection**

138 Rhizon soil solution samplers (5 cm long; Rhizosphere Research Products, Wageningen,
139 Netherlands) were inserted into soil under mosses (mostly *S. uncinata* and *C. aciphyllum*) or
140 vascular plants (*D. antarctica* with some *C. quitensis*). Soil solution was sampled over a depth
141 of *c*. 2–6 cm at approximately fortnightly intervals for about 12 weeks during austral summer.
142 Large soluble proteins and peptides were then removed by passing solutions through a 1 kDa
143 ultrafiltration membrane (Millipore, Billerica, MA, USA).

144 **Analysis of amino acid enantiomers**

145 Filtered soil solution samples taken over the season from each site were pooled, divided in two
146 and concentrated by freeze drying. One portion was hydrolysed for 16 h in 6 M HCl under N_2
147 and freeze-dried again. The dry soil solution residues were re-suspended in 500 µl of 0.01 M
148 HCl with 1.875 pmol μl^{-1} of L-homoarginine as the internal standard. Amino acid enantiomers
149 were quantified by HPLC (Broughton et al. 2015).

150 **Substrate uptake in intact plant-soil system**

151 Monoliths (*c*. 20 × 20 cm) of *D. antarctica* or *C. quitensis* growing in native soil were collected
152 from the Moss Braes region of Signy Island and stored outside for about 24 h prior to

153 experiments. About 1–2 h prior to experiments, 15 mm diameter, 40 mm deep plugs were taken
154 from the monoliths. Solutions (2.5 ml) of 98 at% ^{15}N (inorganic) or dual ^{15}N, ^{13}C (organic) 1
155 mM L-alanine, D-alanine, L-dialanine, D-dialanine, L-trialanine, L-tetraalanine, L-pentaalanine,
156 NH_4Cl or KNO_3 (L-enantiomers, and inorganic from CK-Gas Products, Hook, UK; D-
157 enantiomers from Sigma-Aldrich, Gillingham, UK) were injected into plugs (n=4 and n=3 for
158 *D. antarctica* or *C. quitensis*, respectively). After 1 h in daylight at *c*. 2 ºC, shoot material was
159 removed, dried (80 ºC) and ground before analysis in a Eurovector Isoprime IRMS (Eurovector
160 SpA, Milan, Italy).

161 **Sterile culture of *D. antarctica* and inoculation of roots with DSEs**

162 Sterile individuals of *D. antarctica* (we were not able to generate a sterile culture of *C.*
163 *quitensis*) were prepared according to a protocol modified from Cuba et al. (2005). Plants were
164 removed from soil and washed in tap water. Roots and shoots were trimmed and the remaining
165 tissue was shaken in $NaHClO_3$ (*c*.14% free Cl) with 1 drop of Tween 20 for 25 min, followed
166 by 80% ethanol for 5 min. After thorough washing in sterile tap water, remaining leaf and root
167 was trimmed from crown tissue, which was then placed on the surface of sterile agar containing
168 2.1 g l^{-1} Murashige & Skoog basal medium, 1 mmol l^{-1} glucose and 47 µmol l^{-1} $NaSiO_3$ in
169 Phytatrays (Sigma-Aldrich, Gillingham, UK). Amphotericin B solution (5 ml of 2.5 mg l^{-1})
170 was then added to the surface of agar around the crown tissue. Plants were grown at 10°C with
171 a 16 h photoperiod at *c*. 500 µmol photons m^{-2} s^{-1}. Tillers were separated periodically and re-
172 planted in agar as above (except for amphotericin B, which was not used after the first culture).
173 Any Phytatrays showing signs of microbial contamination were discarded. Examination of
174 roots of sterilised plants by light microscopy and TEM did not reveal the presence of any
175 microbes.

176 Sterile plants for use in experiments were transplanted into Phytatrays containing sterile perlite
177 with *c*. 100 ml of 2.1 g l^{-1} Murashige & Skoog basal medium, 1 mmol l^{-1} glucose and 47 µmol

178 l^{-1} $NaSiO_3$ with and without inoculation with a DSE (*Tapesia* sp.; Helotiales; GenBank
179 accession #FN178471) which was isolated from roots of *D. antarctica* growing on Coronation
180 Island, around 7 km from where experimental plants and soils were collected. At least three
181 weeks was allowed for the DSE to colonise roots before plants were used in experiments. Plants
182 were then removed from the inoculated perlite and grown in uninoculated perlite, as used for
183 the controls.

184 **Substrate uptake from sterile solution**

185 Sterile or DSE-inoculated *D. antarctica* plants were removed from perlite and roots gently
186 washed in sterile 0.1 mM KCl, followed by de-ionised water. Roots of intact plants (*n*=4) were
187 then placed in sterile vials containing 2 ml of 100 µM, 98 at% ^{15}N (inorganic) or dual ^{15}N, ^{13}C
188 (organic) L-alanine, D-alanine, L-dialanine, D-dialanine, L-trialanine, D-trialanine, L-
189 tetraalanine, L-pentaalanine, NH_4Cl or KNO_3. After 1 h, plants were removed from solutions,
190 washed in de-ionised water followed by 100 mM $CaCl_2$. Roots and shoots were separated and
191 analysed by IRMS, as above.

192 **Plant metabolism of substrates**

193 To determine whether substrates could be metabolised, sterile or DSE-inoculated roots of intact
194 *D. antarctica* plants (*n*=3) were submerged in 2 ml of 10 µM, *c*. 7.5 kBq ml^{-1} 1-^{14}C L-alanine,
195 D-alanine, L-dialanine, D-dialanine, L-trialanine, D-trialanine, L-tetraalanine or L-pentaalanine
196 (American Radiolabeled Chemicals, St Louis, MO, USA). Vials and plants were sealed in 50
197 ml clear polypropylene containers. Air was drawn through containers at 300 ml min^{-1} and
198 bubbled through 15 ml Oxysolve C-400 Scintillant (Zinsser Analytic, Frankfurt, Germany) to
199 capture respired $^{14}CO_2$. Carbon dioxide traps were changed after 10, 20, 40, 60 and 80 min and
200 captured $^{14}CO_2$ measured by scintillation counting in a Wallac 1404 scintillation counter
201 (Perkin-Elmer Life Sciences, Waltham, MA, USA).

202 After 80 min, plants were removed from solutions, washed as above and dried. Dry roots and
203 shoots were combusted in a Harvey OX400 Biological Oxidiser (Harvey Instruments Corp.,
204 Hillsdale, NJ, USA). Liberated $^{14}CO_2$ was captured in Oxysolve C-400 and ^{14}C activity
205 measured by liquid scintillation counting as above.

206 **Uptake kinetics**

207 Sterile or DSE-inoculated roots of intact *D. antarctica* plants (n=3) were submerged in labelled
208 (^{14}C or ^{15}N for organic and inorganic substrates, respectively) substrate solutions as above. In
209 this case, exposure to solutions was for 15 min and substrate concentrations were 1, 5, 10, 50,
210 100, 250, 500, 750 µM and 1, 2.5, 5, 7.5 and 10 mM. Plants were analysed for ^{14}C or ^{15}N as
211 above. Respired $^{14}CO_2$ was captured in Oxysolve C-400 and measured as above. Michaelis-
212 Menten constants were calculated from hyperbolic fits to uptake data (Sigmaplot v13, Systat,
213 Hounslow, UK).

214 **NanoSIMS analysis**

215 Sterile or DSE colonised *D. antarctica* (n=3) roots were submerged in 3 mM solution of either
216 $^{13}C^{15}N$ D-trialanine or $^{13}C^{15}N$ -L-trialanine. Plants were incubated for 5 mins then removed
217 from isotope enriched solution, washed quickly in MQ water, then high pressure frozen (HPF;
218 1 mm segments) in hexadecene cryoprotectant (EM PACT2, Leica Microsystems, Wetzlar,
219 Germany). HPF samples were cryosubstituted (EM AFS2, Leica Microsystems, Wetzlar,
220 Germany) using the method described in Bougoure et al. (2014). Briefly, samples were
221 immersed in prechilled (-130 °C) acrolein:diethyl ether over molecular sieve and brought to
222 room temperature over 3 weeks before being infiltrated and embedded in epoxy resin. Sections
223 250 nm thick were cut dry (i.e. not floated onto water for collection), mounted on Si wafers,
224 and Au coated (10 nm) for nanoSIMS analysis. Regions of interest were identified and imaged
225 at 120 kV in a transmission electron microscope (TEM; JEOL 2100) fitted with a digital camera
226 (Gatan, ORIUS1000; Gatan Inc., Pleasanton, CA, USA). Sections were also collected on glass

227 slides, stained with toluidine blue and examined by optical microscopy to guide locations of
228 nanoSIMS analyses.
229 *In situ* isotopic mapping was done using a NanoSIMS 50 (Cameca, Gennevilliers, France),
230 with a 16 keV Cs^{+} primary ion beam. Analyses were performed in multi-collection mode
231 simultaneously detecting negative secondary ions $^{12}C_2$, $^{12}C^{13}C$, $^{12}C^{14}N$, and $^{12}C^{15}N$. The mass
232 spectrometer was tuned to high mass resolution of c. 10000 (CAMECA definition) to separate
233 $^{12}C^{15}N$ from $^{13}C^{14}N$ using an entrance slit of 30 µm, an aperture slit of 200 µm, and a 10%
234 reduction in the signal at the energy slit. For secondary ion imaging, the primary current was
235 set to c. 2 pA using a 350-µm primary aperture, giving a spot size of c. 100 nm. Analyses were
236 done in chain mode so individual 30 × 30 µm analyses (256 pixel resolution) could be
237 montaged to generate a dataset across entire root sections. All areas were implanted to the
238 same ion dose (6×10^{16} ions cm^{-2}) prior to each acquisition.
239 Images were processed using the OpenMIMS data analysis software (National Resource for
240 Imaging Mass Spectrometry http://nrims.harvard.edu) for the freeware package ImageJ
241 (National Institutes of Health, Bethesda, MD, USA). Images were corrected for detector dead
242 time (44 ns) on individual pixels and montages were produced using NRRD mosaics script
243 (http://nrims.harvard.edu).
244 **Statistical analyses**
245 Data were analysed by *t*-test, one-way ANOVA with Tukey HSD post-hoc test or repeated
246 measures ANOVA (SPSS v22; IBM, New York, USA) after testing for normality and
247 homogeneity of variance with Shapiro-Wilk and Levene's tests, respectively. Data not
248 conforming were transformed prior to analysis. Where a suitable transformation could not be
249 identified, Games-Howell test was used. Statistical differences were accepted at $P \leq 0.05$ unless
250 otherwise stated.
251

252

253 **Results**

254 **Amino acid concentrations in soil solution**

255 The presence of vascular plants was associated with increases ($P \leq 0.05$) in soil solution
256 concentrations of 16 out of 18 measured free amino acids (L-enantiomers and glycine) by as
257 much as ten-fold compared to sites where mosses grew alone (Fig. 2). The concentrations of
258 non-protein D-amino acids were more variable, but there was more than three times as much
259 free D-alanine, D-glutamate, D-histidine and D-threonine ($P \leq 0.05$) in soil with vascular plants
260 compared to moss-only soil (the concentrations of three other D-amino acids were greater with
261 statistical significance at $P < 0.1$). Soluble, peptide-bound amino acids tended to be present in
262 soil solution at concentrations approximately ten-times greater than free amino acids
263 (statistically different at $P \leq 0.05$ for 20 and 21 amino acid enantiomers under vascular plants
264 and mosses, respectively). The concentrations of almost half of the bound L-amino acids and
265 D-alanine and D-histidine were greater ($P \leq 0.05$) when vascular plants were present, relative
266 to mosses alone.

267 **Uptake of amino acids and peptides under field conditions**

268 Tests of uptake of a range of N forms under field conditions in the Antarctic showed that both
269 native vascular plant species could acquire ^{15}N from D-alanine and its dipeptide - as well as
270 from peptides of the L-enantiomer up to five amino acids in length (Fig. 3). Rates of uptake
271 appeared similar between the two species. Recovery of amino acid and peptide ^{13}C suggested
272 some intact uptake of molecules, although lack of data for root material and losses of ^{13}C in
273 respiration prevented quantification (Fig. S5 in Supporting Information). Although DSEs were
274 present in the roots of plants used in these experiments, whether the fungal endophytes
275 influenced nutrient acquisition could not be established.

276 **Uptake, partitioning and metabolism of amino acids and peptides by plants with sterile**
277 **roots or colonised with DSEs**

278 Although there were minor differences between isotopic tracers, with the exception of nitrate,
279 DSE colonisation increased the uptake of all forms of N supplied to roots, with strong positive
280 effects of the endophyte on the uptake of L-tri-, L-tetra- and L-pentaalanine ($P < 0.05$; Fig. 4).
281 Nitrate was also the only tested form of N where Michaelis-Menten constants for N uptake
282 showed no indication of an effect of DSE colonisation (Table S1 in Supporting Information).
283 Surprisingly, the DSE appeared to promote N translocation such that colonised plants had a
284 lower ratio of root ^{15}N to shoot ^{15}N than uninoculated control plants ($P < 0.001$; Fig. S6 in
285 Supporting Information). Further, in contrast to limited data for other plants, loss of $^{14}CO_2$ in
286 respiration demonstrated that *D. antarctica* could metabolise all forms of organic N supplied,
287 including D-peptides (Fig. S7 in Supporting Information; Hill et al. 2011c). However, actual
288 rates of C loss in respiration are probably somewhat overestimated due to the ^{14}C label being
289 located only on the carboxyl group (Dippold & Kuzyakov 2013; Hill & Jones 2019).
290 Nanoscale Secondary Ion Mass Spectrometry (nanoSIMS) showed transfer of L-peptide ^{15}N
291 into the intercellular space between the root cortical cells of *D. antarctica* by DSE hyphae (Fig.
292 5; Fig. S8 in Supporting Information). Additionally, individual root cells of plants supplied
293 with D- or L-trialanine were more enriched with ^{15}N when colonised with the DSE than in
294 sterile controls, strongly suggesting that enhanced isotope recovery in bulk root analyses was
295 not merely separate uptake by roots and fungus.
296

297 **Discussion**

298 It appears that the presence of vascular plants in the organic soils of the maritime Antarctic
299 gives rise to a marked increase in availability of both L- and D-enantiomers of amino acids as
300 N sources. This suggests a stimulation of the rate of breakdown of stored moss peat in the

301 presence of roots, probably resulting from rhizosphere priming (Gavazov et al. 2018). Of free
302 (and peptide-bound) D-amino acids, D-alanine was amongst the most available, maintaining
303 concentrations around 10% of those of L-alanine, despite microbial consumption at rates
304 similar to those of L-amino acids, indicating a significant production flux in these soils (Hill et
305 al. 2011b). Whether this D-alanine originates primarily from peptidoglycan, abiotic
306 racemisation of L-alanine in stored proteins, or another process is currently unknown.
307 Similarly, although we can attribute occurrence of other D-amino acids to racemisation, it is
308 not clear whether this is the only or even the principal source (Vranova et al. 2012). However,
309 irrespective of the exact origin, the actual increase in availability of amino acid-N driven by
310 vascular plants is likely to be greater than the increase in measured soil solution concentrations,
311 due to a probable higher consumption flux from both microbes and plant roots in soils under
312 vascular plants than under mosses (Hill et al. 2011a,b).
313 DSEs are widespread in plant roots in a range of ecosystems (Jumpponen 2001; Newsham et
314 al. 2008), but there has been limited identification of their roles in plant nutrient acquisition to
315 date, with some appearing to have negative effects on plant hosts (Jumpponen 2001; Upson et
316 al. 2009; Newsham 2011; Vergara et al. 2018). Consequently, it remains unknown whether
317 symbioses with DSEs are widespread facilitators of nutrient acquisition. It is clear from the
318 findings here that the colonisation of roots by DSEs has a marked effect on the ability of
319 Antarctic angiosperms to exploit amino acid N. The nanoSIMS images demonstrate direct
320 hyphal transfer of peptide N to the root, and the surprising effect of DSE colonisation on
321 translocation of N suggests an additional physiological effect on the host plant (direct hyphal
322 transfer to shoots is unlikely due to confinement of this group of fungi to roots; Rodriguez et
323 al. 2009). Colonisation appears to aid acquisition of some forms of N, such as peptides of D-
324 amino acids and an L-pentapeptide, which have not previously been recognised as viable
325 sources of N for plants. This may be due to the probable higher availability of both L- and D-

326 enantiomers in ecosystems where large quantities of proteinaceous material accumulate and
327 turn over slowly (Chapin et al. 1993; Kunnas & Jauhiainen 1993; Wichern et al. 2004). The
328 occurrence of close relatives of the DSE used here in the Arctic may support this view
329 (Genbank accessions MF920427 and KF617231; Krishnan et al. 2018; Taylor et al. 2014).
330 However, as both D- and L-peptides do exist in other ecosystems and investigation into plant
331 use of D-peptide N has been limited, it may be that the use of these N forms by both plants and
332 DSEs is more widespread than is currently recognised (Friedman 2010; Hill et al. 2011c;
333 Vranova et al. 2012). Some mosses are also colonised by endophytic fungi, but there is no
334 evidence for a role of these endophytes in nutrient acquisition (Davey & Currah 2006).
335 As greenhouse gas emissions to the atmosphere continue, near-surface air temperatures in the
336 maritime Antarctic are projected to warm by 2–4 °C by 2100 (Bracegirdle et al. 2008). Our
337 measurements suggest that vascular plants could increase rates of organic matter breakdown
338 under Antarctic mosses by up to an order of magnitude. Rising air temperatures are known to
339 synergistically increase rhizosphere priming, with increases in temperature sensitivity of,
340 perhaps, 25-50% in the presence of living roots (Boone et al. 1998; Zhu & Cheng 2011; Hill et
341 al. 2015). Hence, it appears that priming of ancient organic matter stored in moss banks arising
342 from plant growth and warming may interact to further increase nutrient availability, enhancing
343 the proliferation of angiosperms and returning more C to the atmosphere in a complex positive
344 feedback (Convey & Smith 2006; Day et al. 2008; Cannone et al. 2016; Gavazov et al. 2018;
345 Newsham et al. 2018). Thus, it seems probable that the stocks of moss-derived organic matter
346 accumulated over millennia will disappear at increasingly rapid rates as temperatures rise and
347 the ecology of the maritime Antarctic changes.
348

349 **References**

350 Abram, N.J. et al. (2013). Acceleration of snow melt in an Antarctic Peninsula ice core during
351 the twentieth century. *Nat. Geosci.,* 6, 404–411.
352 Amesbury, M.J. et al. (2017).Widespread biological response to rapid warming on the
353 Antarctic Peninsula. *Curr. Biol*., 27, 1616-1622.
354 Boone, R.D., Nadelhoffer, K.J., Canary, J.D. & Kaye, J.P. (1998). Roots exert a strong
355 influence on the temperature sensitivity of soil respiration. *Nature*, 396, 570–572.
356 Bougoure, J. et al. (2014). High resolution secondary ion mass spectrometry analysis of carbon
357 dynamics in mycorrhizas formed by an obligately myco-heterotrophic orchid. *Plant Cell*
358 *Environ.*, 37, 1223-1230.
359 Bracegirdle, T.J., Connolley, W.M. & Turner, J. (2008). Antarctic climate change over the
360 twenty first century. *J. Geophys. Res*., 113, D03103
361 Broughton, R.C.I., Newsham, K.K., Hill, P.W., Stott, A. & Jones, D.L. (2015). Comparison of
362 the incorporation of D- and L-enantiomeric forms of alanine and its peptides into PLFAs by
363 different components of an Antarctic soil microbial community. *Soil Biol. Biochem*., 88, 83-
364 89.
365 Cannone, N., Guglielmin, M., Convey, P., Worland, M.R. & Favero Longo, S.E. (2016).
366 Vascular plant changes in extreme environments: effects of multiple drivers. *Clim. Change,*
367 134, 651-665.
368 Chapin, F.S., Moilanen, L. & Kielland, K. (1993). Preferential use of organic nitrogen for
369 growth by a non-mycorrhizal arctic sedge. *Nature,* 361, 150-153.
370 Convey, P. & Smith, R.I.L. (2006). Responses of terrestrial Antarctic ecosystems to climate
371 change. *Plant. Ecol.,* 182, 1-10.
372 Cuba, M., Gutiérrez-Moraga, A., Butendieck, B. & Gidekel, M. (2005). Micropropogation of
373 *Deschampsia antarctica* – a frost-resistant Antarctic plant. *Antarct. Sci*., 17, 69-70.

374 Day, T.A., Ruhland, C.T. & Xiong, F.S. (2008). Warming increases aboveground plant
375 biomass and C stocks in vascular-plant-dominated Antarctic tundra. *Glob. Change Biol.,* 14,
376 1827-1843.
377 Dippold, M.A. & Kuzyakov, Y. (2013). Biogeochemical transformations of amino acids in soil
378 assessed by position-specific labelling. *Plant Soil*, 373, 385-401.
379 Friedman, M. (2010) Origin, microbiology, nutrition and pharmacology of D-amino acids.
380 *Chem. Biodivers*. 7, 1491-1530.
381 Gavazov, K. et al. (2018). Vascular plant-mediated controls on atmospheric carbon
382 assimilation and peat carbon decomposition under climate change. *Glob. Change Biol*., 24,
383 3911-3921.
384 Hill, P.W. et al. (2015). Living roots magnify the response of soil organic carbon
385 decomposition to temperature in temperate grassland. *Glob. Change Biol*., 21, 1368-1375.
386 Hill, P.W. et al. (2011a). Vascular plant success in a warming Antarctic may be due to efficient
387 nitrogen acquisition. *Nat. Clim. Change,* 1, 50-53.
388 Hill, P.W., Farrell, M. & Jones, D.L. (2012). Bigger may be better in soil N cycling: does rapid
389 acquisition of small L-peptides by soil microbes dominate fluxes of protein-derived N in soil?
390 *Soil Biol. Biochem*., 48, 106-112.
391 Hill, P.W. et al. (2011b). Soil- and enantiomer-specific metabolism of amino acids and their
392 peptides by Antarctic soil microorganisms. *Soil Biol. Biochem.,* 43, 2410-2416.
393 Hill, P.W. et al. (2011c). Acquisition and assimilation of nitrogen as peptide-bound and D-
394 enantiomers of amino acids by wheat. *PLoS ONE,* 6, e19220.
395 Hill, P.W. & Jones, D.L. (2019). Plant-microbe competition: does injection of isotopes of C
396 and N into the rhizosphere effectively characterise plant use of soil N? *New Phytol.* 221, 796-
397 806.

398 Jumpponen, A. (2001). Dark septate endophytes – are they mycorrhizal? *Mycorrhiza,* 11, 207-
399 211.
400 Krishnan, A., Convey, P., Gonzalez, M., Smykla, J. & Alias, S.A. (2018). Effects of
401 temperature on extracellular hydrolase enzymes from soil microfungi. *Polar Biol.*, 41, 537-
402 551.
403 Kunnas, A.V. & Jauhiainen, T.-P. (2011). Separation and identification of free amino acid
404 entantiomers in peat by capillary gas chromatography. *J. Chromatogr*. **628**, 269-273 (1993).
405 Newsham, K.K. A meta-analysis of plant responses to dark septate root endophytes. *New*
406 *Phytol.*, 190, 783–793.
407 Newsham, K. K. & Bridge, P.D. (2010). Sebacinales are associates of the leafy liverwort
408 Lophozia excisa in the southern maritime Antarctic. *Mycorrhiza*, 20, 307–313.
409 Newsham, K.K., Garnett, M.H., Robinson, C.H. & Cox, F. (2018). Discrete taxa of
410 saprotrophic fungi respire different ages of carbon from Antarctic soils. *Sci. Rep.*, 8, 7866.
411 Newsham, K.K., Upson, R. & Read, D.J. (2009). Mycorrhizas and dark septate root endophytes
412 in polar regions. *Fungal Ecol.*, 2, 10-20.
413 Rodriguez, R.J., White, J.F., Arnold, A.E. & Redman, R.S. (2009). Fungal endophytes:
414 diversity and functional roles. *New Phytol*. 182, 314-330.
415 Royles, J. & Griffiths, H. (2015). Climate change impacts in polar regions: lessons from
416 Antarctic moss bank archives. *Glob. Change Biol.*, 21, 1041-1057.
417 Royles, J. et al. (2012). Carbon isotope evidence of recent climate-related enhancement of CO_2
418 assimilation and peat accumulation rates in Antarctica. *Glob. Change Biol.*, 18, 3112-3124.
419 Smith, S.E. & Read, D.J. (2008). *Mycorrhizal Symbiosis*. Academic press.
420 Strullu-Derrien, C., Selosse, M.-A., Kenrick, P. & Martin, F.M. (2018). The origin and
421 evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytol.,*
422 220, 1012-1030.

423 Taylor, L.D., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C. & Ruess,
424 R.W. (2014). A first comprehensive census of fungi in soil reveals both hyperdiversity and
425 fine-scale niche partitioning. *Ecol. Monogr*., 84, 3-20.

426 Upson, R., Newsham, K.K. & Read, D.J. (2008). Root-fungal associations of *Colobanthus*
427 *quitensis* and *Deschampsia antarctica* in the Maritime and Subantarctic. *Arct. Antarct. Alp.*
428 *Res.,* 40, 592-599.

429 Upson, R., Read, D.J. & Newsham, K.K. (2009). Nitrogen form influences the response of
430 *Deschampsia antarctica* to dark septate root endophytes. *Mycorrhiza,* 20, 1-11.

431 Vergara, C. et al. (2018). Dark septate endophytic fungi increase green manure-^{15}N recovery
432 efficiency, N contents and micronutrients in rice grains. *Front. Plant Sci*., 9, 613.

433 Vranova, V. et al. (2012). The significance of D-amino acids in soil, fate and utilization by
434 microbes and plants: review and identification of knowledge gaps. *Plant Soil,* 354, 21-39.

435 Wichern, F. et al. (2004). Changes in amino acid enantiomers and microbial performance in
436 soils from a subtropical mountain oasis in Oman abandoned for different periods. *Biol. Fert.*
437 *Soils,* 39, 398-406.

438 Yoshimura, T. & Esaki, N. (2003). Amino acid racemases; functions and mechanisms. *J.*
439 *Biosci. Bioeng.* 96, 103-109.

440 Zhu, B. & Cheng, W. (2011). Rhizosphere priming effect increases the temperature sensitivity
441 of soil organic matter decomposition. *Glob. Change Biol*., 17, 2172-2183.

442

443

444 **Supporting Information**

445 Additional Supporting Information may be downloaded via the online version of this article at
446 Wiley Online Library (www.ecologyletters.com).

447

448 **Acknowledgements**

449 We thank Stacey Adlard and Bruce Maltman for assistance with fieldwork, Liam Smallwood,
450 Peter Talboys and Sarah Chesworth for laboratory assistance, Rebecca Upson and British
451 Antarctic Survey staff who are too numerous to mention by name. Special thanks to Jonathan
452 and Linda Roberts. The authors acknowledge the facilities of Microscopy Australia at the
453 Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, a
454 facility funded by the University, State and Commonwealth Governments. This work was
455 funded by UK Natural Environment Research Council grant NE/I012303/1.

456

457 **Figures**

458 **Figure 1** Antarctic vascular plants exploiting areas previously colonised by mosses on Signy
459 Island and DSEs in roots of *Deschampsia antarctica*. a. *D. antarctica* growing in a bank of
460 *Chorisodontium aciphyllum*. b. *D. antarctica* growing through mixed *Sanionia uncinata* and
461 *Polytrichum juniperinum*. c. *D. antarctica* growing amongst *Andreaea* sp. d. *Colobanthus*
462 *quitensis* growing through *C. aciphyllum*. e. *C. quitensis* growing through *S. uncinata*. f. *D.*
463 *antarctica* and *C. quitensis* growing with *S. uncinata*. g. DSE hyphae in *D. antarctica* root. h.
464 DSE microsclerotium (arrowed) in *D. antarctica* root (scale bars on panels g and h are 20 µm).
465

466 **Figure 2** Concentrations of D- and L-enantiomers of amino acids in soil solutions at Signy
467 Island under mosses alone or where vascular plants are present. a. free amino acids. b. amino
468 acids bound in soluble peptides. Values are means ± SEM; *n*=23 and *n*=16 for free and bound
469 amino acids, respectively, under vascular plants; *n*= 26 and *n*=21 for free and bound amino
470 acids, respectively, under mosses only. Asterisks indicate differences between soil where
471 vascular plants are present or where mosses are present alone ($P \leq 0.05$).
472

473 **Figure 3** Rates of uptake of inorganic N and D-and L-enantiomers of alanine and short peptides
474 thereof into shoots of *D. antarctica* and *C. quitensis* following injection of ^{15}N- and ^{13}C-
475 labelled substrates into soil. Values are mean ± SEM; *n*=3 or 4.
476

477 **Figure 4** Rates of uptake by *D. antarctica* of N supplied in different forms. N uptake calculated
478 from recovery of ^{14}C (a) and ^{15}N (b). Data are mean ± SEM; *n*=3 and *n*=4 for ^{14}C and ^{15}N,
479 respectively. Calculation of N flux from ^{14}C assumes that C and N entered the plant (or plant
480 and fungus) together without extracellular separation of C and N. ^{13}C data did not account for
481 respiratory losses and are not shown.

482

483 **Figure 5** ^{15}N distribution within *D. antarctica* roots with and without DSE colonisation after 5
484 min incubation in either D or L enantiomers of ^{15}N trialanine. a. Optical image of partial DSE-
485 inoculated root cross-section showing typical cell zonation, specifically the cortex (white inset
486 square) from where nanoSIMS images (c) are taken; scale bar 100 µm. b. TEM of intercellular
487 space between root cortical cells of a DSE-inoculated root showing the presence of abundant
488 hyphae (white arrows); scale bar 2 µm. c. The ^{15}N atom percent images (nanoSIMS) of typical
489 cortical cells in roots with or without DSE and incubated with either D or L forms of ^{15}N
490 trialanine. Highest ^{15}N enrichment was observed in DSE colonised roots supplied with L-
491 trialanine. White arrows indicate intercellular hyphae where they can be clearly identified.
492 Cells of DSE colonised roots supplied with D-trialanine also showed enrichment, but hyphae
493 could not be located with confidence. Roots without DSE showed negligible ^{15}N enrichment;
494 scale bar 10 µm.

495

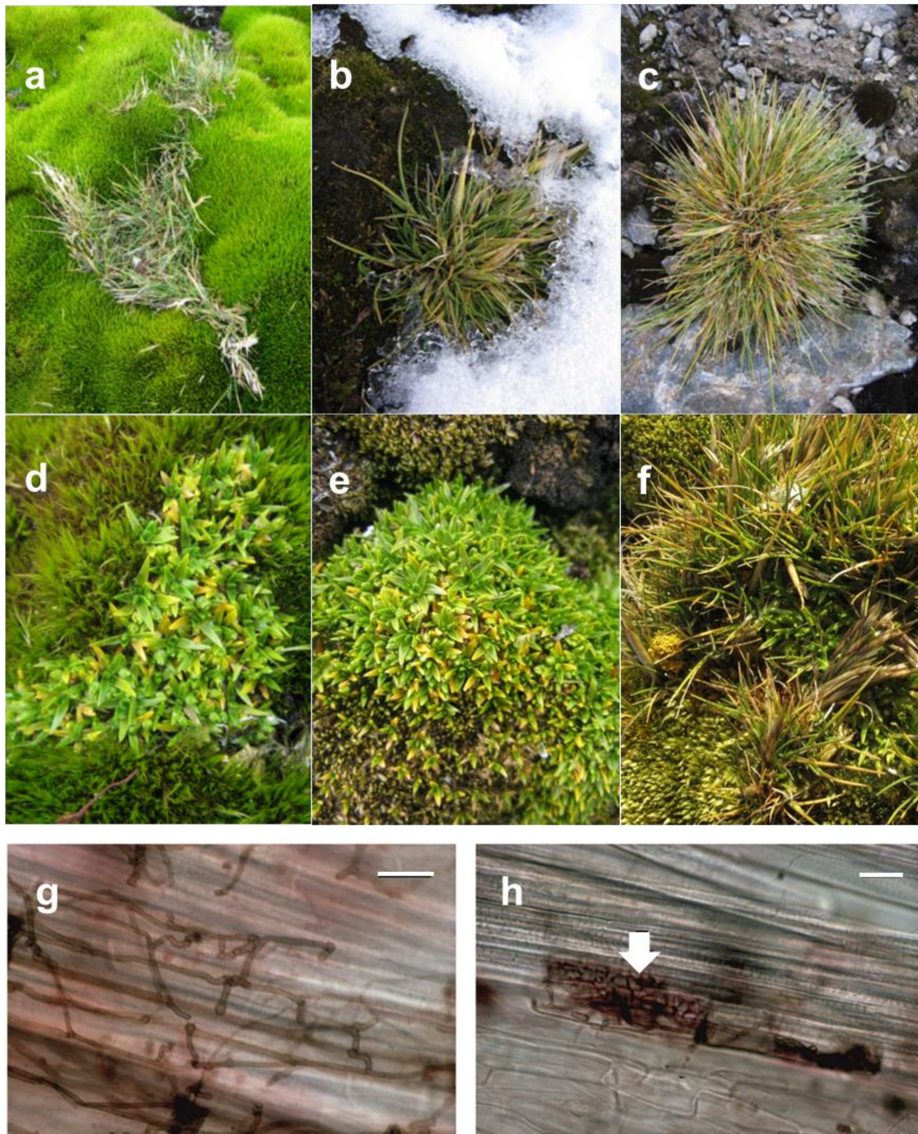
496

497

498

499

500



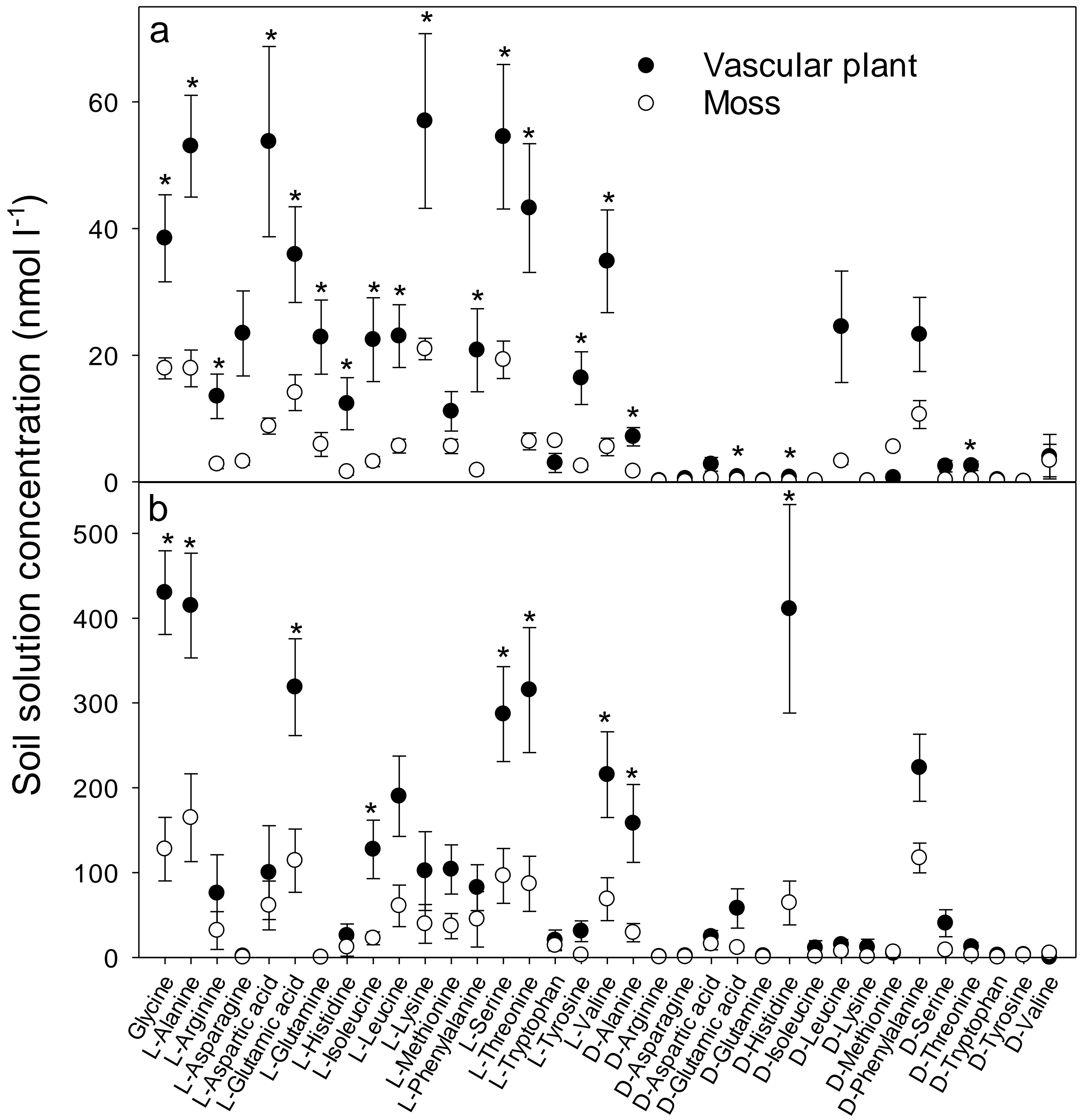
501

502 **Figure 1**

503

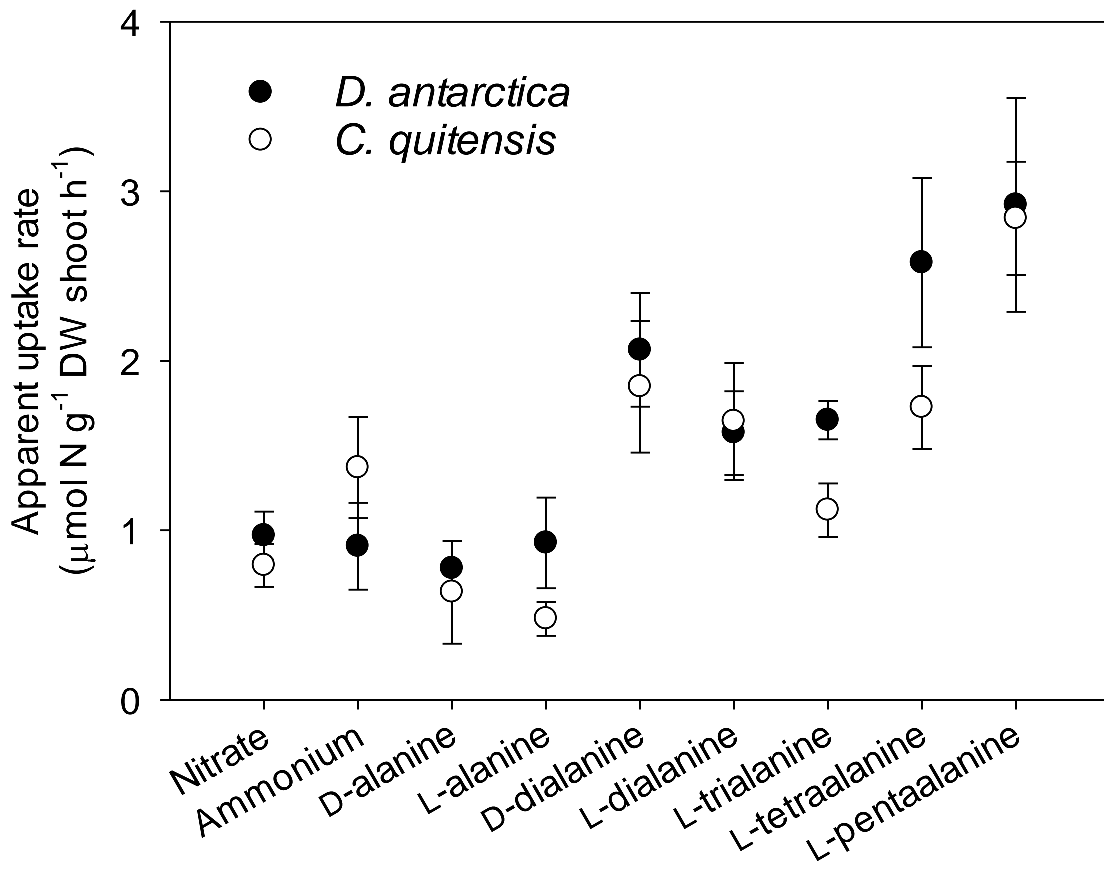
504

505



506 **Figure 2**

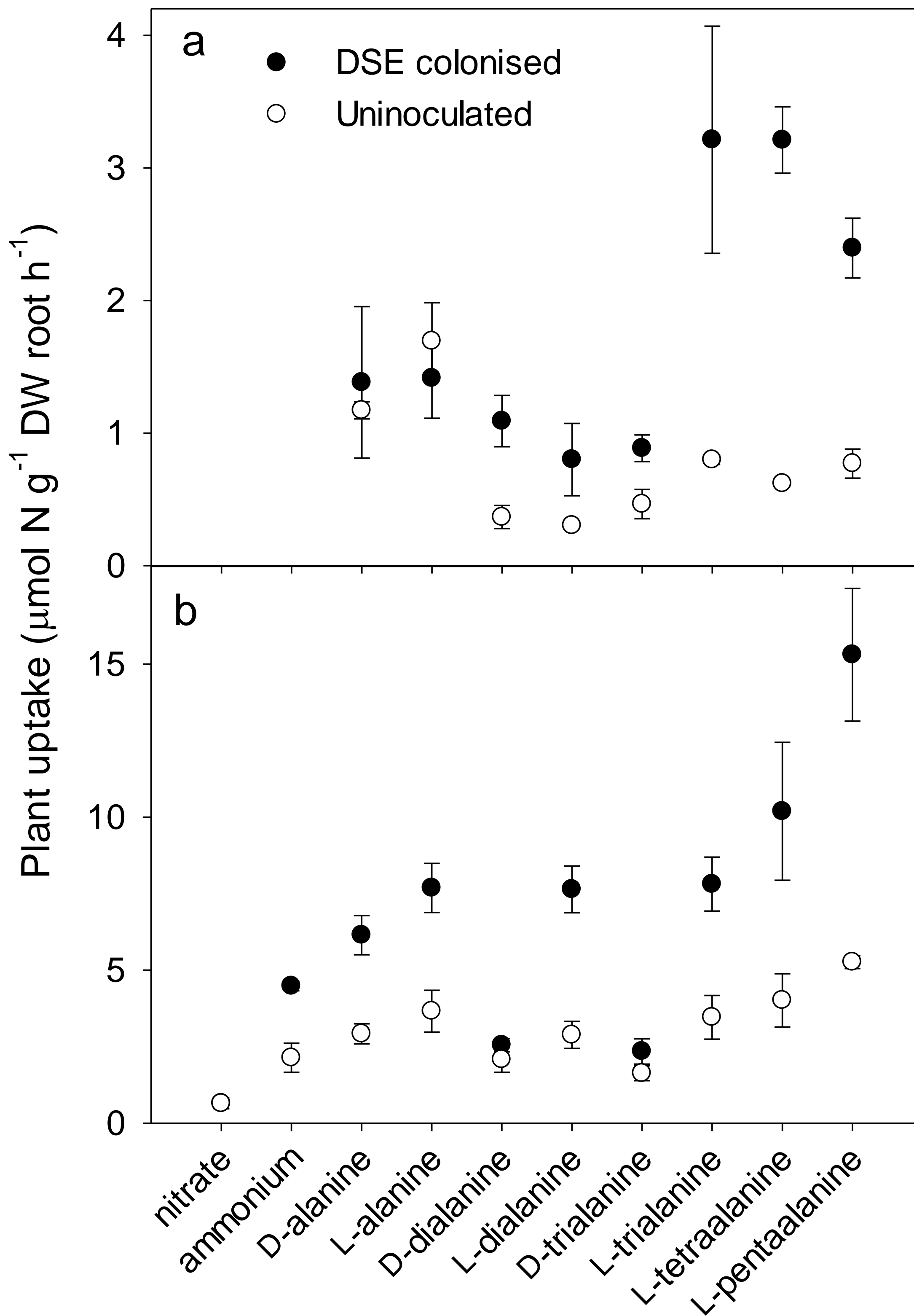
507



508

509 **Figure 3**

510

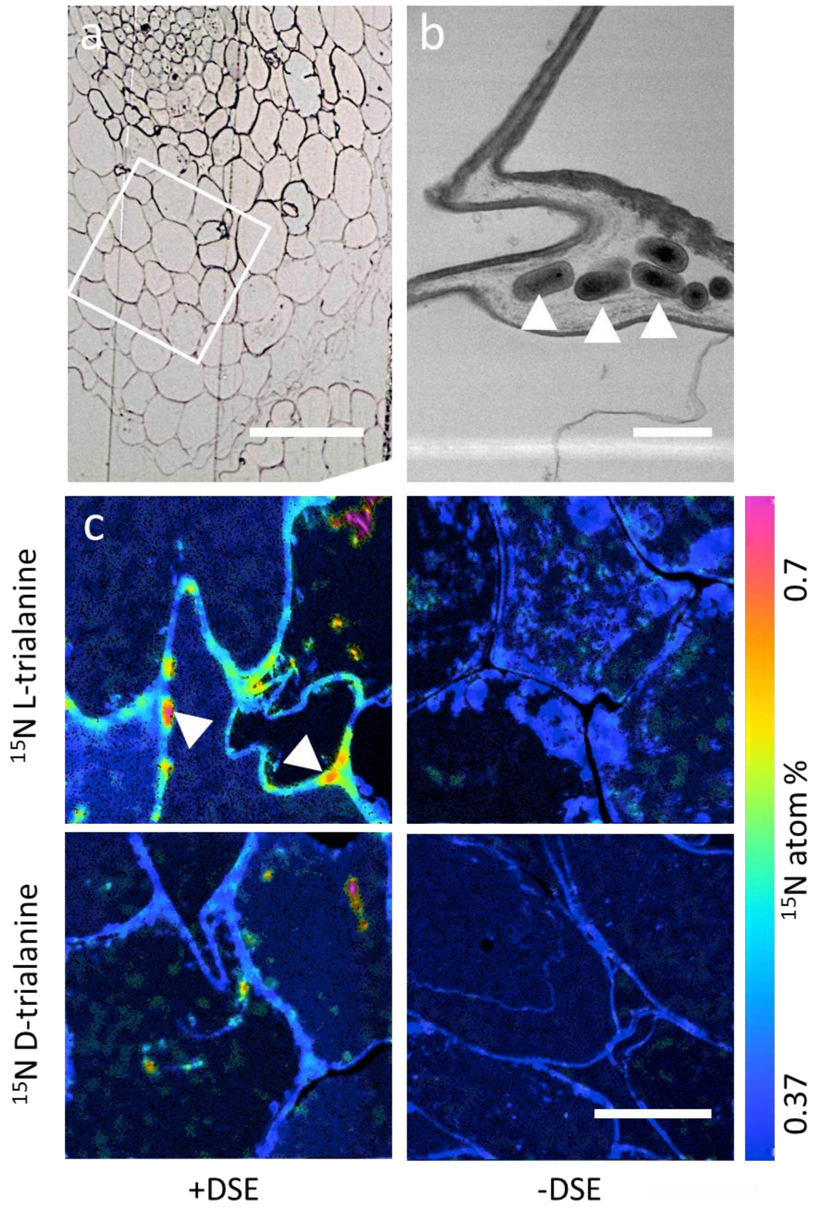


511

512 **Figure 4**

513

514



515

516 **Figure 5**

517

518

519

520

521

522

523

524 **Figure S1** Moss carpet dominated by *Sanionia uncinata* on Signy Island.

525

526

527

528

529

530

531

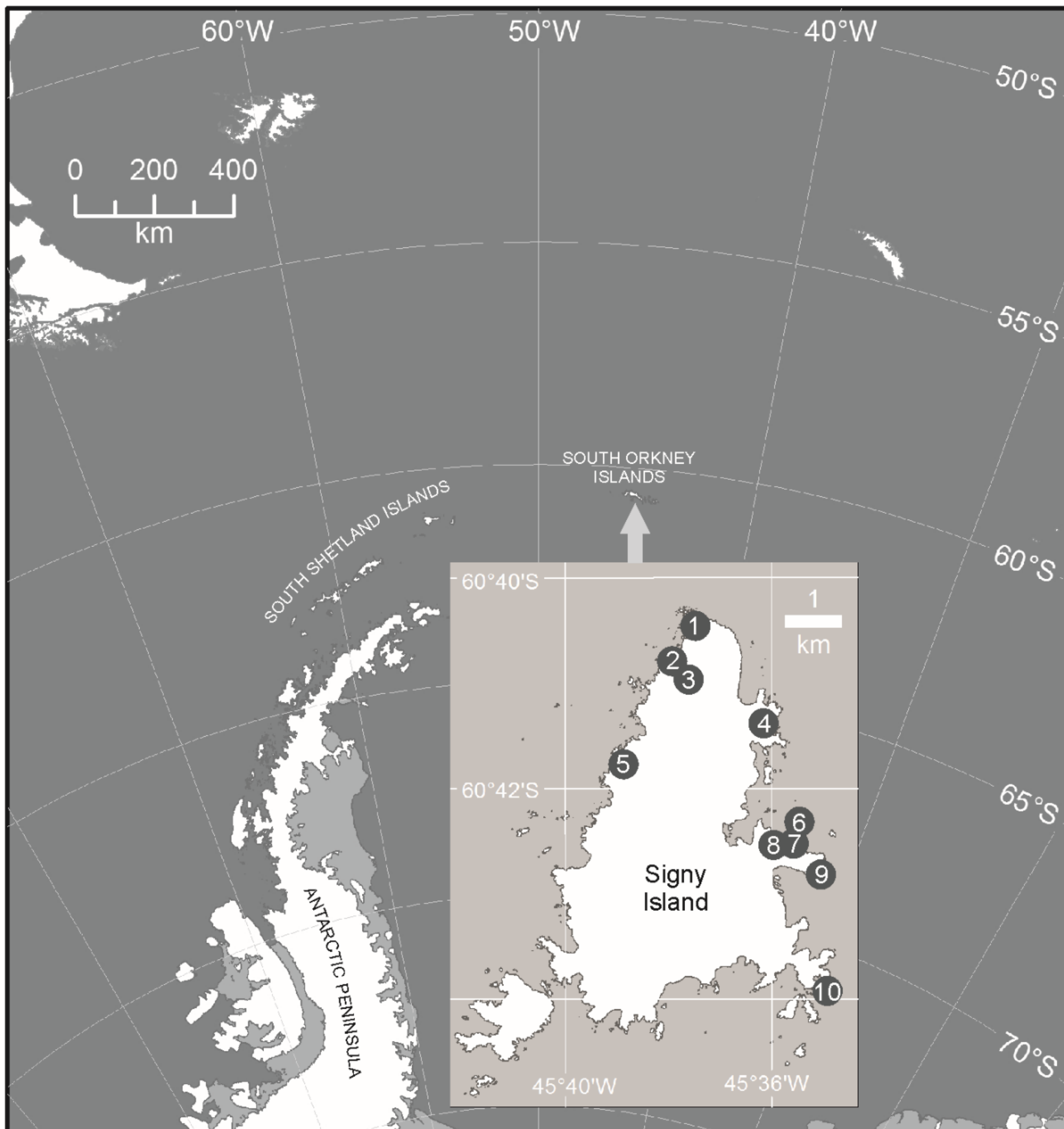
532 **Figure S2** Moss banks on Signy Island, showing living *Chorisodontium aciphyllum* with
533 accumulated moss peat underneath.
534

535

536

537

538 **Figure S3** *Deschampsia antarctica* with shallow roots penetrating into accumulated organic
539 matter under *Sanionia uncinata*. Pencil gives scale (*c*. 6 mm diameter).
540
541
542



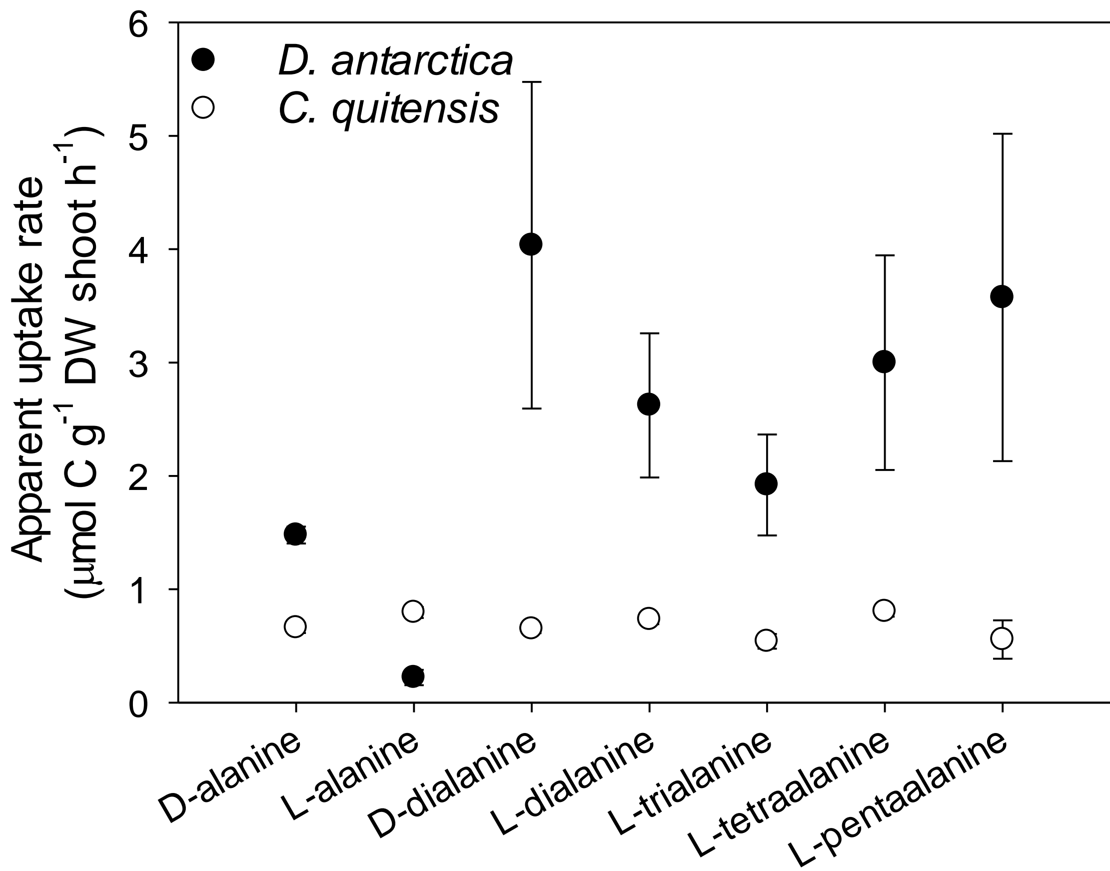
543

544 **Figure S4** Location of Signy Island (inset) in maritime Antarctica. Plants were sampled from (1)

545 North Point, (2) Deschampsia Point, (3) Moss Braes, (4) Starfish Cove, (5) Foca Cove, (6)

546 Berntsen Point, (7) Factory Cove, (8) Factory Bluffs, (9) Polynesia Point and (10) Gourlay Point.

547

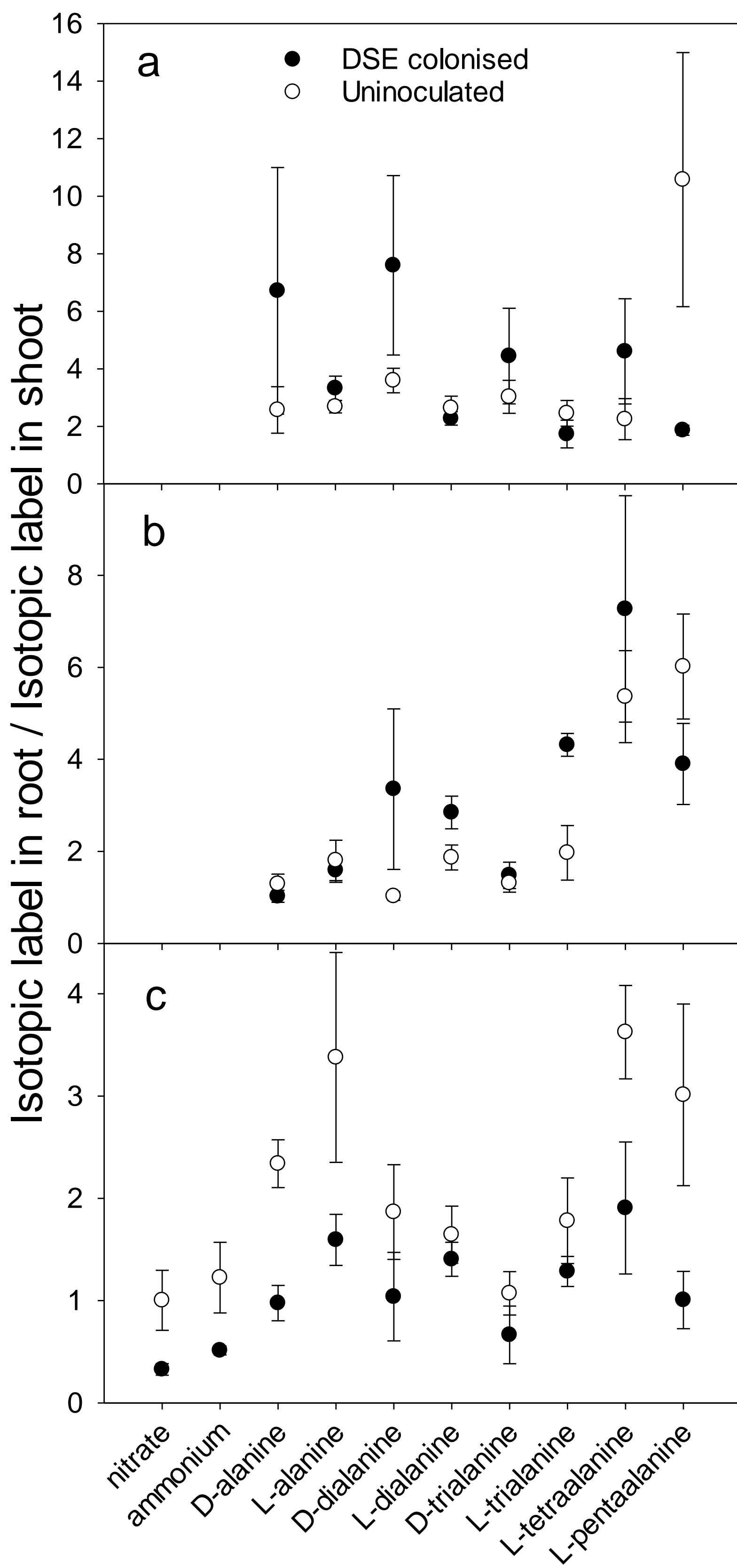


548

549

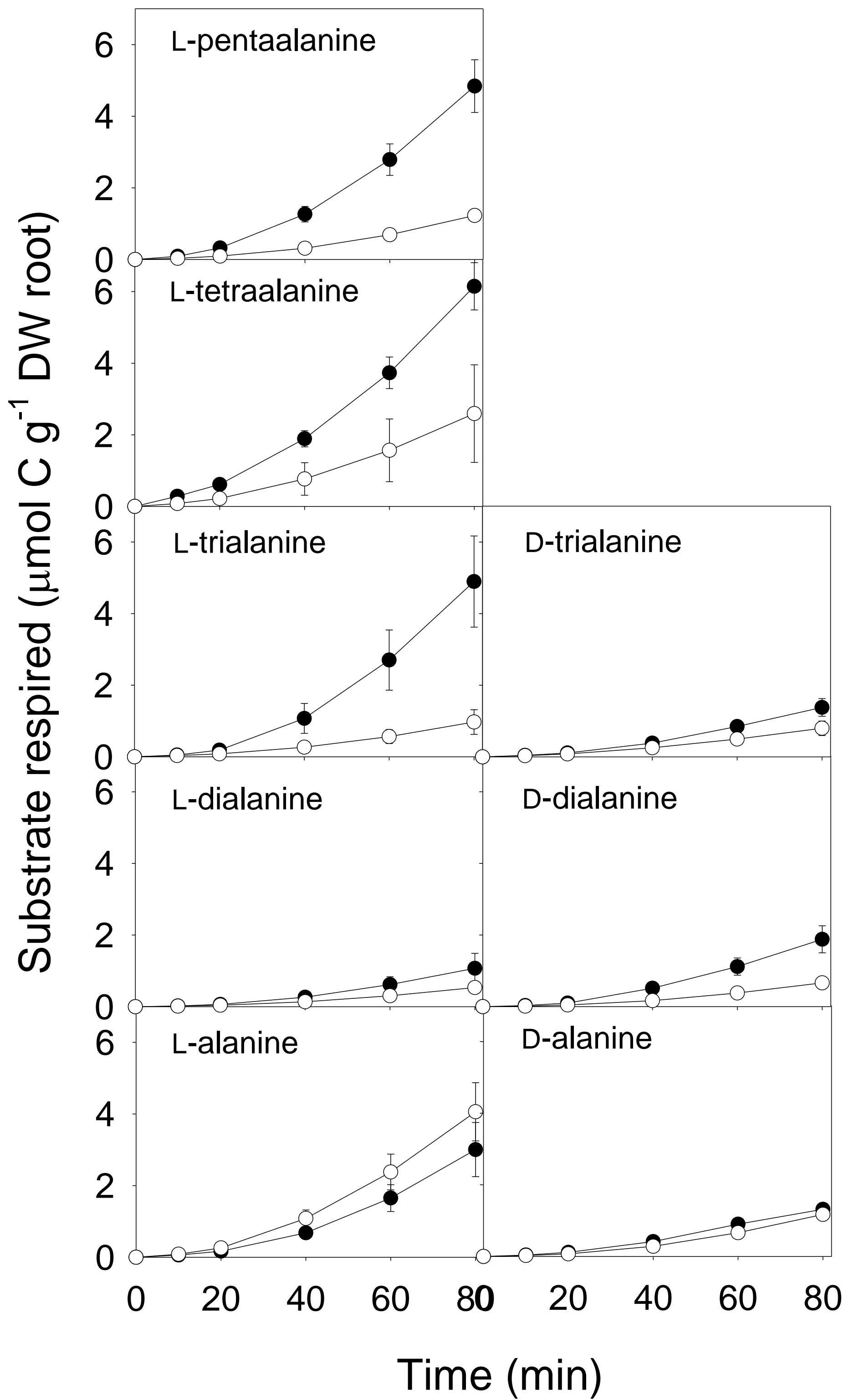
550 **Figure S5** Apparent rates of uptake of C from D-and L-enantiomers of alanine and short peptides
551 thereof into shoots of *D. antarctica* and *C. quitensis* following injection of ^{15}N- and ^{13}C- labelled
552 substrates into soil. Values are mean ± SEM; *n*=3 or 4. Caution should be exercised in
553 interpretation as differences in partitioning and losses of ^{13}C in respiration are not accounted for.
554

555



556

557 **Figure S6** Ratio of recovery of isotopic labels in roots to recovery in shoots of *D. antarctica*. a.
558 ^{14}C. b. ^{13}C. c. ^{15}N. Data are mean ± SEM; *n*=3 for ^{14}C; *n*=4 for ^{13}C and ^{15}N.

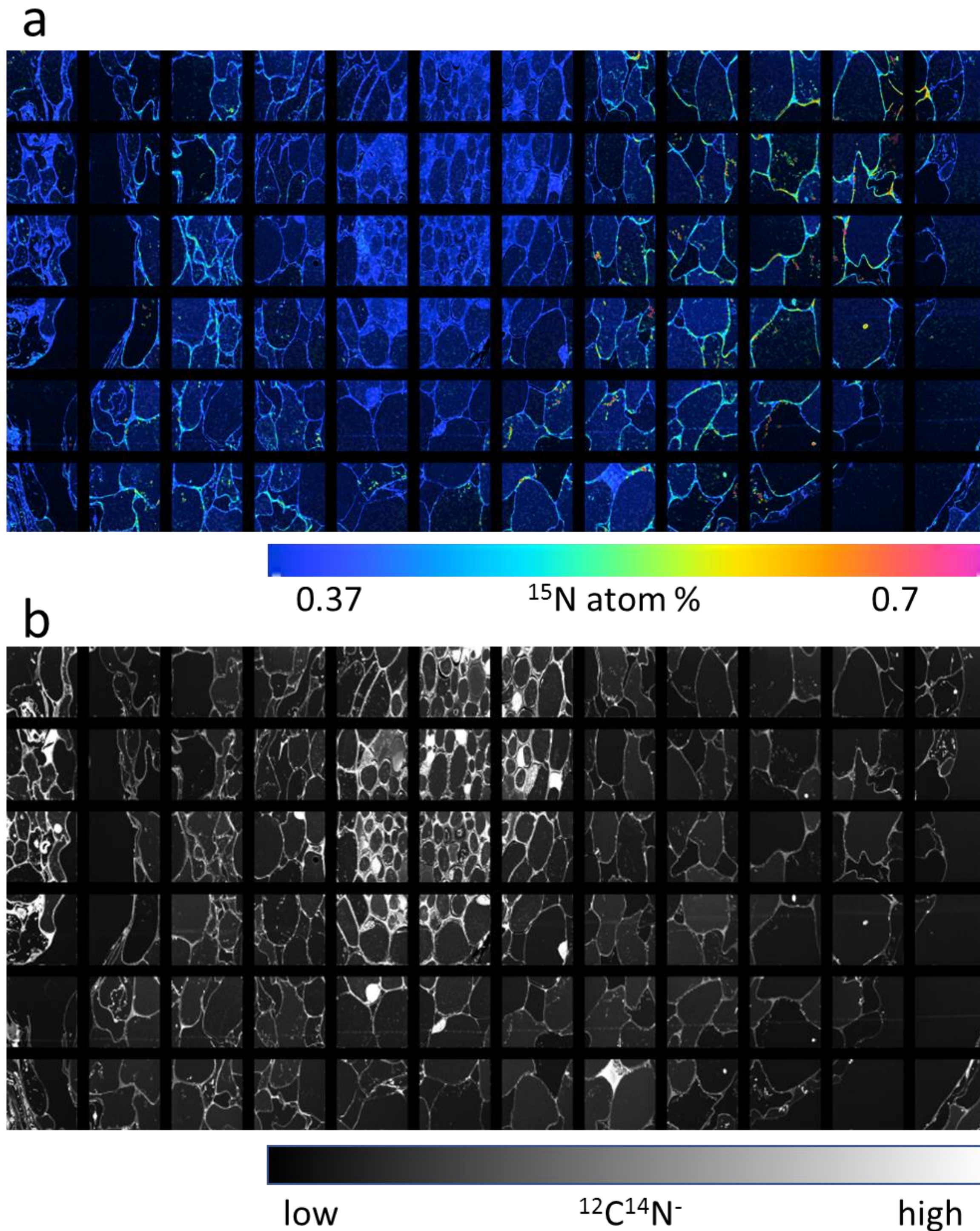


559

560 **Figure S7** Loss in respiration of C supplied to roots of *D. antarctica* as D- and L-alanine and their
561 short peptides. Closed and open circles are plants colonised with DSE and unicolulated controls,
562 respectively. Data are mean ± SEM; *n*=3.

563

564



565

566 **Figure S8** ^{15}N enrichment image of entire root cross-section of *D. antarctica* inoculated with
567 DSE and incubated for 5 min in ^{15}N L-trialanine. Similar montaged images were generated for all
568 four treatments (+/-DSE, D or L ^{15}N-trialinine) and an unlabelled control. For the example
569 displayed here (A), ^{15}N enrichment is highest in the intercellular spaces of the cortical zone and
570 also in portions of microsclerotia. B) $^{12}C^{14}N^-$ ion (proxy for ^{14}N) intensity image of the same area
571 is included as a reference to sample ultrastructure.

572 **Table S1** Michaelis-Menten constants for uptake of various forms of N by roots of *D. antarctica*
573 without or with DSE colonisation

	Km (µmol l^{-1})		Vmax (µmol g^{-1} DW root h^{-1})		Difference between DSE colonised and control plants
	-DSE	+DSE	-DSE	+DSE	
NO_3^-	3488	3308	42.0	36.8	P=0.90
NH_4^+	5743	5191	35.9	42.2	P=0.007
L-alanine	323.1	840.6	12.5	42.7	P=0.07
D-alanine	657.1	782.3	15.2	20.6	P=0.04
L-dialanine	222.2	410.9	11.1	45.3	P=0.02
D-dialanine	1261	663.4	27.0	23.2	P=0.08

574

575

576