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

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

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Abstract

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

Introduction

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

antarctica to exploit ancient nutrients that up until recent decades were unavailable because the moss banks have been frozen.

Due to slow N mineralisation, it is likely that early breakdown products of accumulated proteins (L-amino acids and their short peptides) make a substantial contribution to plant N nutrition in polar soils (Chapin et al. 1993; Hill et al. 2011a). However, peptides containing D-glutamic acid and especially D-alanine are common constituents of bacterial peptidoglycan and various D-amino acids occur in bacteria, archaea, fungi, plants and animals (Yoshimura & Esaki 2003; Friedman 2010; Vranova et al. 2012). D-amino acids are also known to accumulate from proteinaceous L-amino acids during long periods of storage, due to abiotic racemisation, which may take place at a rates of about 0.3% of L-amino acids per decade (Wichern et al. 2004). Consequently, D-amino acids accumulate in soils where decomposition is slow e.g., in deserts or in peat, such as that formed by moss banks (Kunnas & Jauhiainen 1993; Wichern et al. 2004).

It is clear from previous investigations that both plants and soil microbes are able to take up and metabolise some D-amino acids such as D-alanine (Hill et al. 2011b,c; Hill et al. 2012; Vranova et al. 2012). However, in contrast to short L-peptides, which appear to be widely metabolised, until now, evidence suggested that short D-peptides could be metabolised by soil microbes but not by plants (Hill et al. 2011b,c; Hill et al. 2012; Vranova et al. 2012). Whether the ability to metabolise D-peptides is present in plants inhabiting soils where D-enantiomers are a more available source of N is unknown. We measured uptake of a range of N forms under field conditions in the Antarctic and found that both native vascular plants could acquire N from D-alanine and its dipeptide - as well as from longer peptides of the L-enantiomer than previously recognised. Further, we found that colonisation with DSEs facilitated plant acquisition of N from both L- and D-enantiomers of alanine and their peptides.

Materials and Methods

Assessment of fungal endophyte colonisation

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

Soil solution collection

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

Analysis of amino acid enantiomers

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

Substrate uptake in intact plant-soil system

Monoliths (*c*. 20 × 20 cm) of *D. antarctica* or *C. quitensis* growing in native soil were collected 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).

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

Uptake kinetics

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

NanoSIMS analysis

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

slides, stained with toluidine blue and examined by optical microscopy to guide locations of nanoSIMS analyses.

In situ isotopic mapping was done using a NanoSIMS 50 (Cameca, Gennevilliers, France), with a 16 keV Cs^+ primary ion beam. Analyses were performed in multi-collection mode simultaneously detecting negative secondary ions $^{12}C_2$, $^{12}C^{13}C$, $^{12}C^{14}N$, and $^{12}C^{15}N$. The mass spectrometer was tuned to high mass resolution of c. 10000 (CAMECA definition) to separate $^{12}C^{15}N$ from $^{13}C^{14}N$ using an entrance slit of 30 µm, an aperture slit of 200 µm, and a 10% reduction in the signal at the energy slit. For secondary ion imaging, the primary current was set to c. 2 pA using a 350-µm primary aperture, giving a spot size of c. 100 nm. Analyses were done in chain mode so individual 30 × 30 µm analyses (256 pixel resolution) could be montaged to generate a dataset across entire root sections. All areas were implanted to the same ion dose (6×10^{16} ions cm^{-2}) prior to each acquisition.

Images were processed using the OpenMIMS data analysis software (National Resource for Imaging Mass Spectrometry http://nrims.harvard.edu) for the freeware package ImageJ (National Institutes of Health, Bethesda, MD, USA). Images were corrected for detector dead time (44 ns) on individual pixels and montages were produced using NRRD mosaics script (http://nrims.harvard.edu).

Statistical analyses

Data were analysed by *t*-test, one-way ANOVA with Tukey HSD post-hoc test or repeated measures ANOVA (SPSS v22; IBM, New York, USA) after testing for normality and homogeneity of variance with Shapiro-Wilk and Levene's tests, respectively. Data not conforming were transformed prior to analysis. Where a suitable transformation could not be identified, Games-Howell test was used. Statistical differences were accepted at $P \leq 0.05$ unless otherwise stated.

Results

Amino acid concentrations in soil solution

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

Uptake of amino acids and peptides under field conditions

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

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

Although there were minor differences between isotopic tracers, with the exception of nitrate, DSE colonisation increased the uptake of all forms of N supplied to roots, with strong positive effects of the endophyte on the uptake of L-tri-, L-tetra- and L-pentaalanine ($P < 0.05$; Fig. 4). Nitrate was also the only tested form of N where Michaelis-Menten constants for N uptake showed no indication of an effect of DSE colonisation (Table S1 in Supporting Information). Surprisingly, the DSE appeared to promote N translocation such that colonised plants had a lower ratio of root ^{15}N to shoot ^{15}N than uninoculated control plants ($P < 0.001$; Fig. S6 in Supporting Information). Further, in contrast to limited data for other plants, loss of $^{14}CO_2$ in respiration demonstrated that *D. antarctica* could metabolise all forms of organic N supplied, including D-peptides (Fig. S7 in Supporting Information; Hill et al. 2011c). However, actual rates of C loss in respiration are probably somewhat overestimated due to the ^{14}C label being located only on the carboxyl group (Dippold & Kuzyakov 2013; Hill & Jones 2019).

Nanoscale Secondary Ion Mass Spectrometry (nanoSIMS) showed transfer of L-peptide ^{15}N into the intercellular space between the root cortical cells of *D. antarctica* by DSE hyphae (Fig. 5; Fig. S8 in Supporting Information). Additionally, individual root cells of plants supplied with D- or L-trialanine were more enriched with ^{15}N when colonised with the DSE than in sterile controls, strongly suggesting that enhanced isotope recovery in bulk root analyses was not merely separate uptake by roots and fungus.

Discussion

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

presence of roots, probably resulting from rhizosphere priming (Gavazov et al. 2018). Of free (and peptide-bound) D-amino acids, D-alanine was amongst the most available, maintaining concentrations around 10% of those of L-alanine, despite microbial consumption at rates similar to those of L-amino acids, indicating a significant production flux in these soils (Hill et al. 2011b). Whether this D-alanine originates primarily from peptidoglycan, abiotic racemisation of L-alanine in stored proteins, or another process is currently unknown. Similarly, although we can attribute occurrence of other D-amino acids to racemisation, it is not clear whether this is the only or even the principal source (Vranova et al. 2012). However, irrespective of the exact origin, the actual increase in availability of amino acid-N driven by vascular plants is likely to be greater than the increase in measured soil solution concentrations, due to a probable higher consumption flux from both microbes and plant roots in soils under vascular plants than under mosses (Hill et al. 2011a,b).

DSEs are widespread in plant roots in a range of ecosystems (Jumpponen 2001; Newsham et al. 2008), but there has been limited identification of their roles in plant nutrient acquisition to date, with some appearing to have negative effects on plant hosts (Jumpponen 2001; Upson et al. 2009; Newsham 2011; Vergara et al. 2018). Consequently, it remains unknown whether symbioses with DSEs are widespread facilitators of nutrient acquisition. It is clear from the findings here that the colonisation of roots by DSEs has a marked effect on the ability of Antarctic angiosperms to exploit amino acid N. The nanoSIMS images demonstrate direct hyphal transfer of peptide N to the root, and the surprising effect of DSE colonisation on translocation of N suggests an additional physiological effect on the host plant (direct hyphal transfer to shoots is unlikely due to confinement of this group of fungi to roots; Rodriguez et al. 2009). Colonisation appears to aid acquisition of some forms of N, such as peptides of D-amino acids and an L-pentapeptide, which have not previously been recognised as viable sources of N for plants. This may be due to the probable higher availability of both L- and D-

enantiomers in ecosystems where large quantities of proteinaceous material accumulate and turn over slowly (Chapin et al. 1993; Kunnas & Jauhiainen 1993; Wichern et al. 2004). The occurrence of close relatives of the DSE used here in the Arctic may support this view (Genbank accessions MF920427 and KF617231; Krishnan et al. 2018; Taylor et al. 2014). However, as both D- and L-peptides do exist in other ecosystems and investigation into plant use of D-peptide N has been limited, it may be that the use of these N forms by both plants and DSEs is more widespread than is currently recognised (Friedman 2010; Hill et al. 2011c; Vranova et al. 2012). Some mosses are also colonised by endophytic fungi, but there is no evidence for a role of these endophytes in nutrient acquisition (Davey & Currah 2006).

As greenhouse gas emissions to the atmosphere continue, near-surface air temperatures in the maritime Antarctic are projected to warm by 2–4 °C by 2100 (Bracegirdle et al. 2008). Our measurements suggest that vascular plants could increase rates of organic matter breakdown under Antarctic mosses by up to an order of magnitude. Rising air temperatures are known to synergistically increase rhizosphere priming, with increases in temperature sensitivity of, perhaps, 25-50% in the presence of living roots (Boone et al. 1998; Zhu & Cheng 2011; Hill et al. 2015). Hence, it appears that priming of ancient organic matter stored in moss banks arising from plant growth and warming may interact to further increase nutrient availability, enhancing the proliferation of angiosperms and returning more C to the atmosphere in a complex positive feedback (Convey & Smith 2006; Day et al. 2008; Cannone et al. 2016; Gavazov et al. 2018; Newsham et al. 2018). Thus, it seems probable that the stocks of moss-derived organic matter accumulated over millennia will disappear at increasingly rapid rates as temperatures rise and the ecology of the maritime Antarctic changes.

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Supporting Information

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

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456

Figures

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

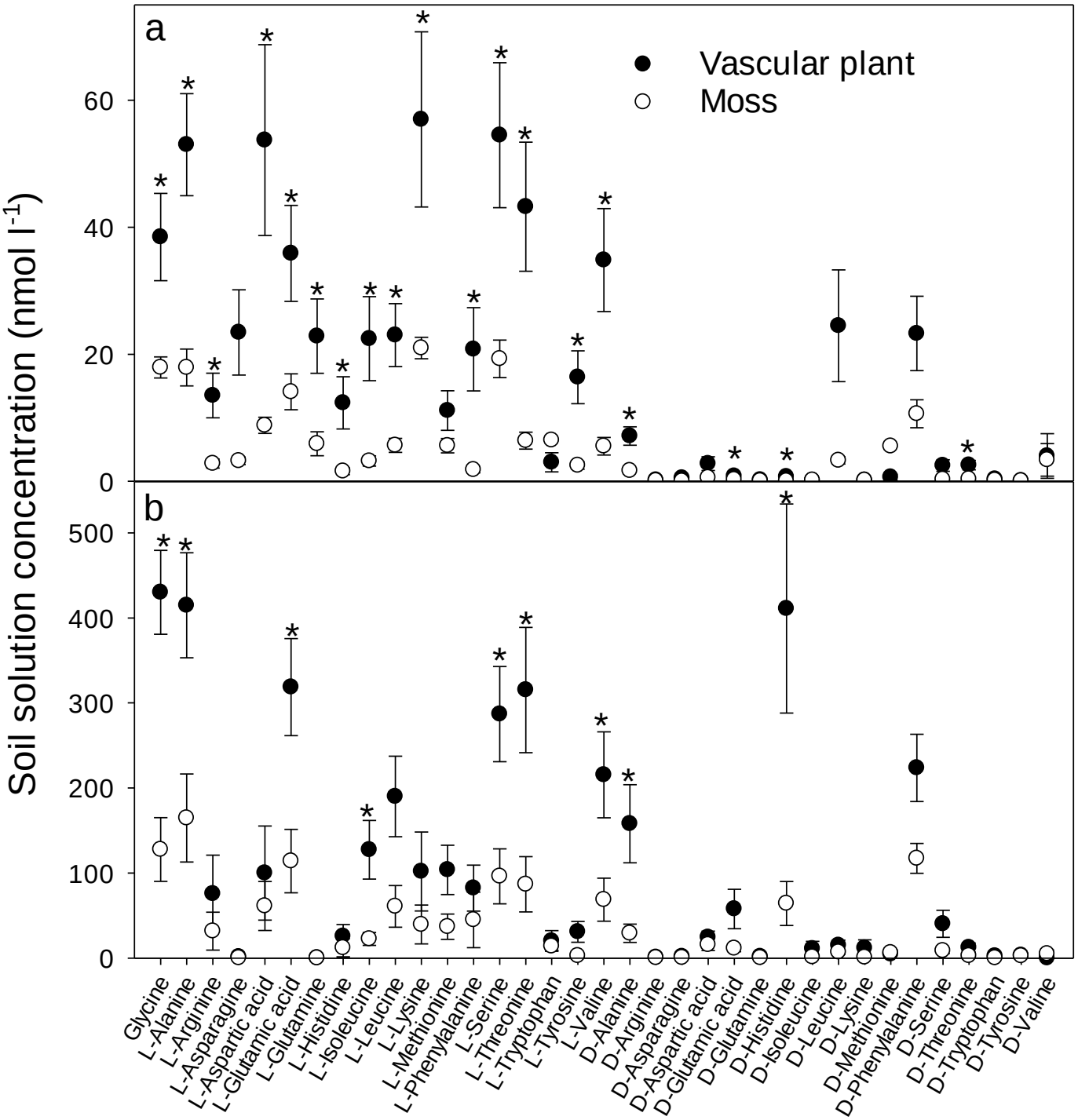
Figure 2 Concentrations of D- and L-enantiomers of amino acids in soil solutions at Signy Island under mosses alone or where vascular plants are present. a. free amino acids. b. amino acids bound in soluble peptides. Values are means ± SEM; n=23 and n=16 for free and bound amino acids, respectively,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

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

Figure 4 Rates of uptake by *D. antarctica* of N supplied in different forms. N uptake calculated 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, respectively. Calculation of N flux from ^{14}C assumes that C and N entered the plant (or plant and fungus) together without extracellular separation of C and N. ^{13}C data did not account for respiratory losses and are not shown.

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

Figure 1



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506 **Figure 2**

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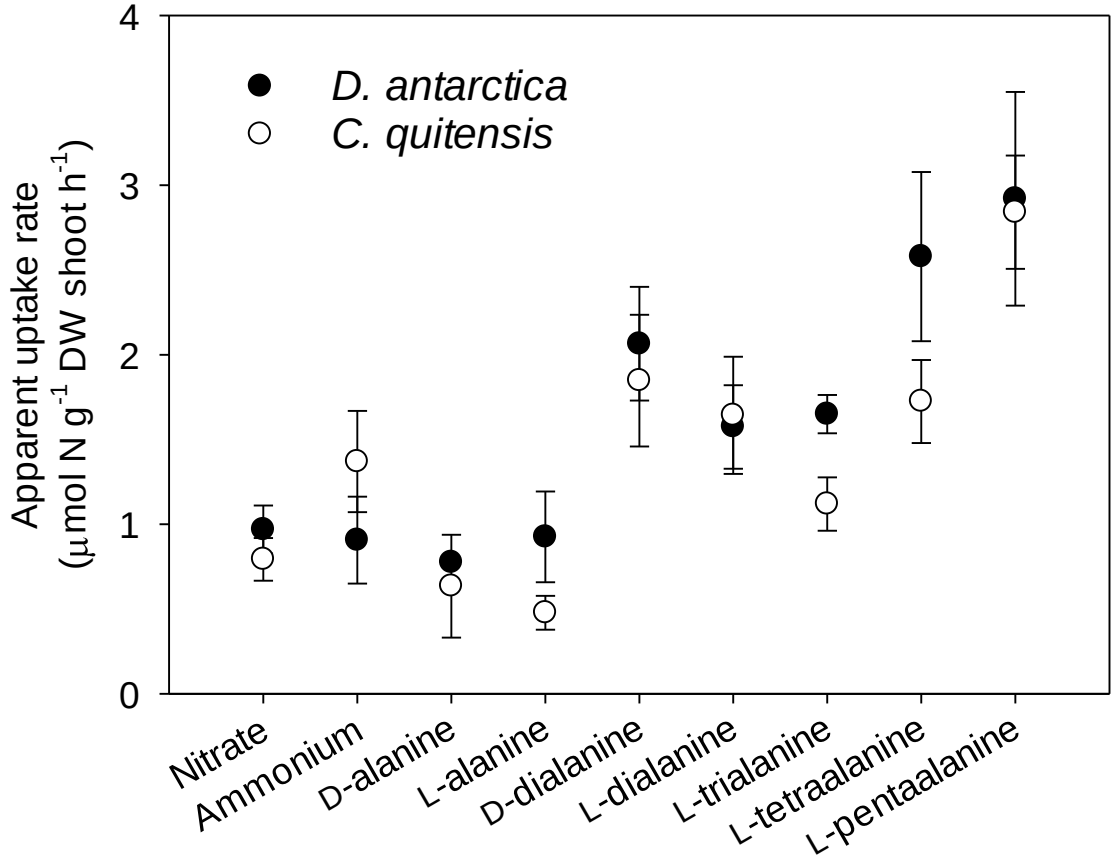

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509 **Figure 3**

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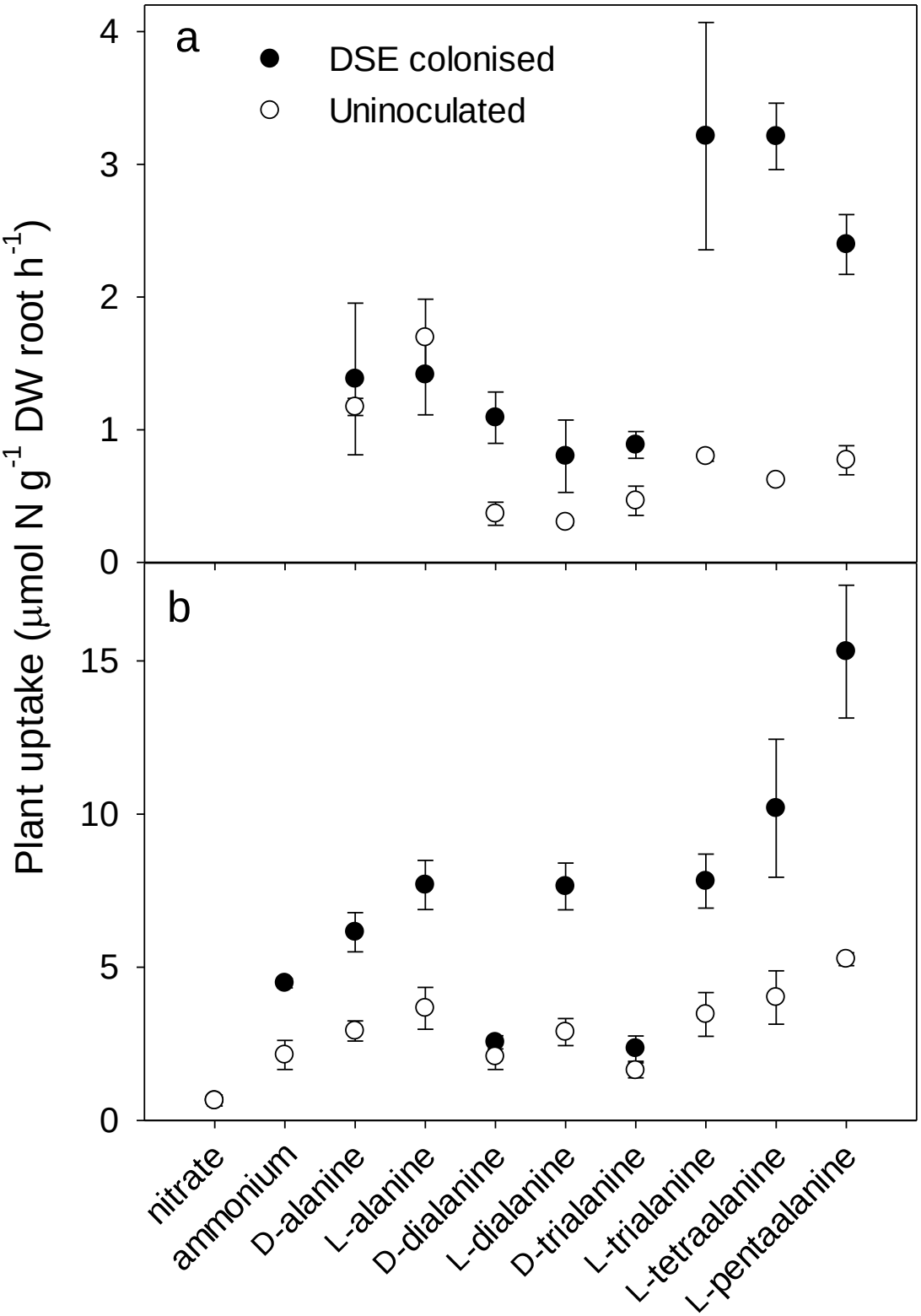

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512 **Figure 4**

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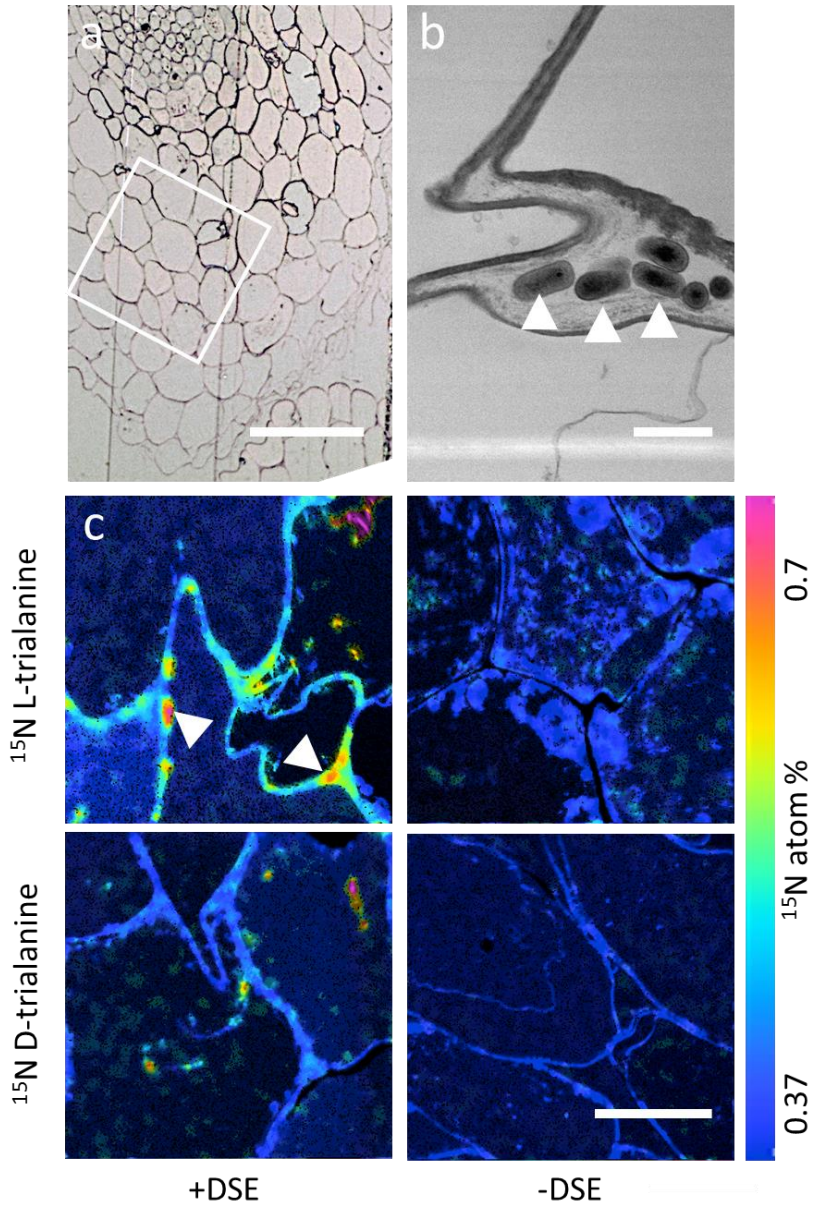


Figure 5

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

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

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

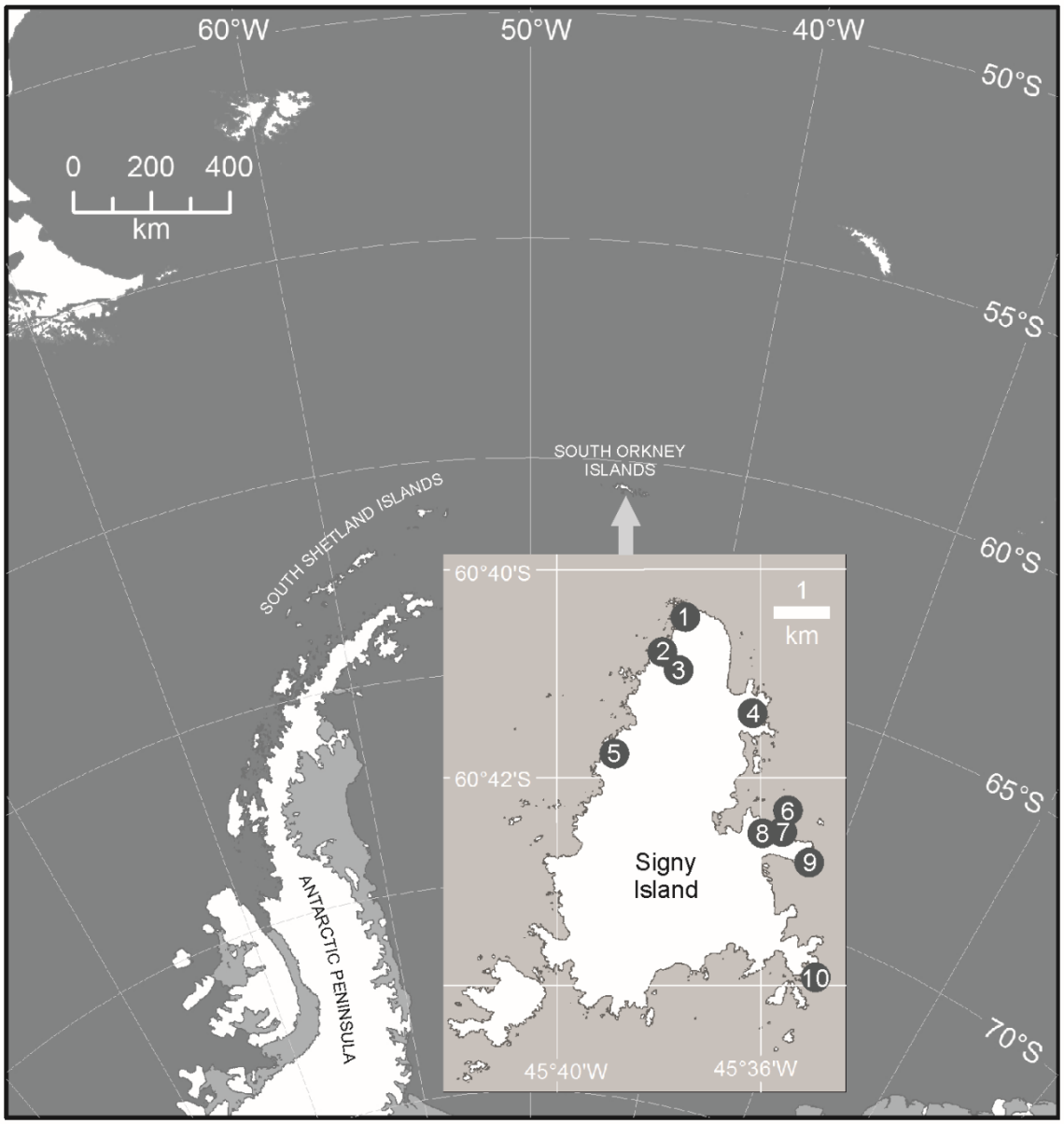
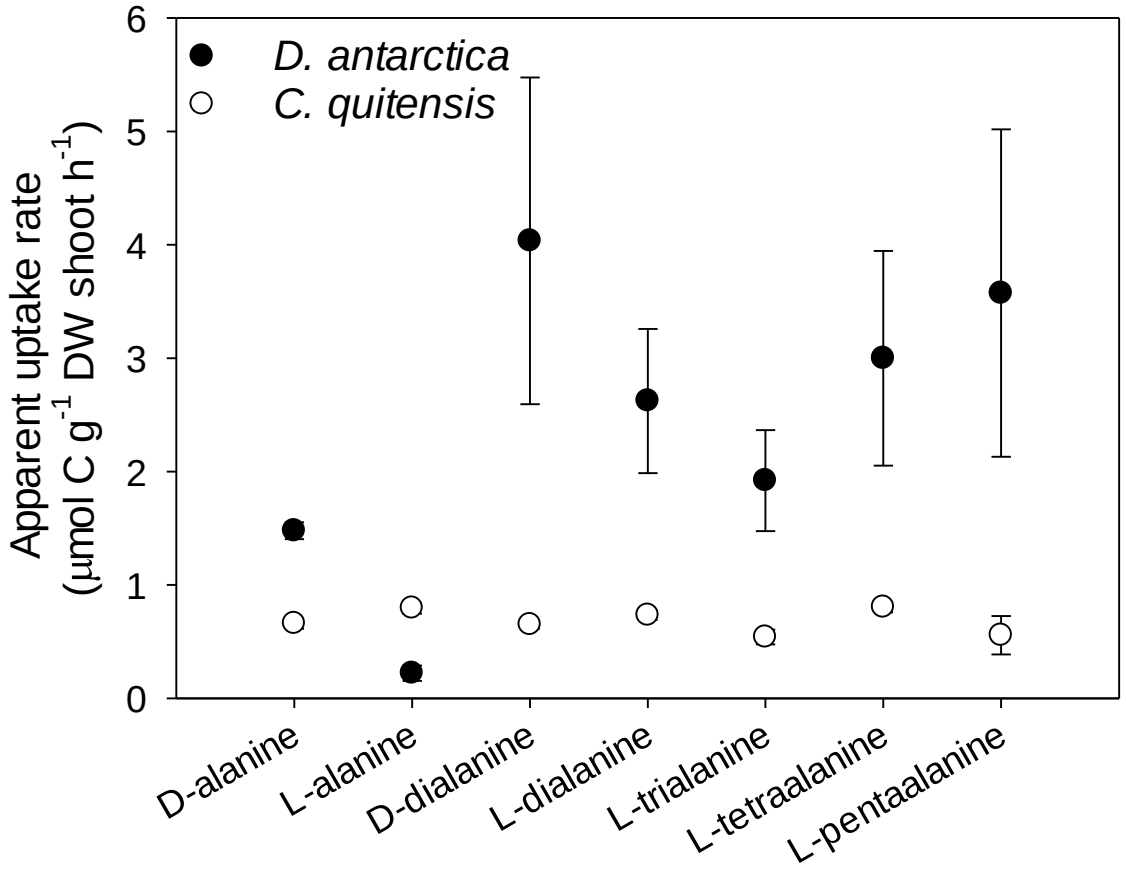


Figure S4 Location of Signy Island (inset) in maritime Antarctica. Plants were sampled from (1) North Point, (2) Deschampsia Point, (3) Moss Braes, (4) Starfish Cove, (5) Foca Cove, (6) Berntsen Point, (7) Factory Cove, (8) Factory Bluffs, (9) Polynesia Point and (10) Gourlay Point.



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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.
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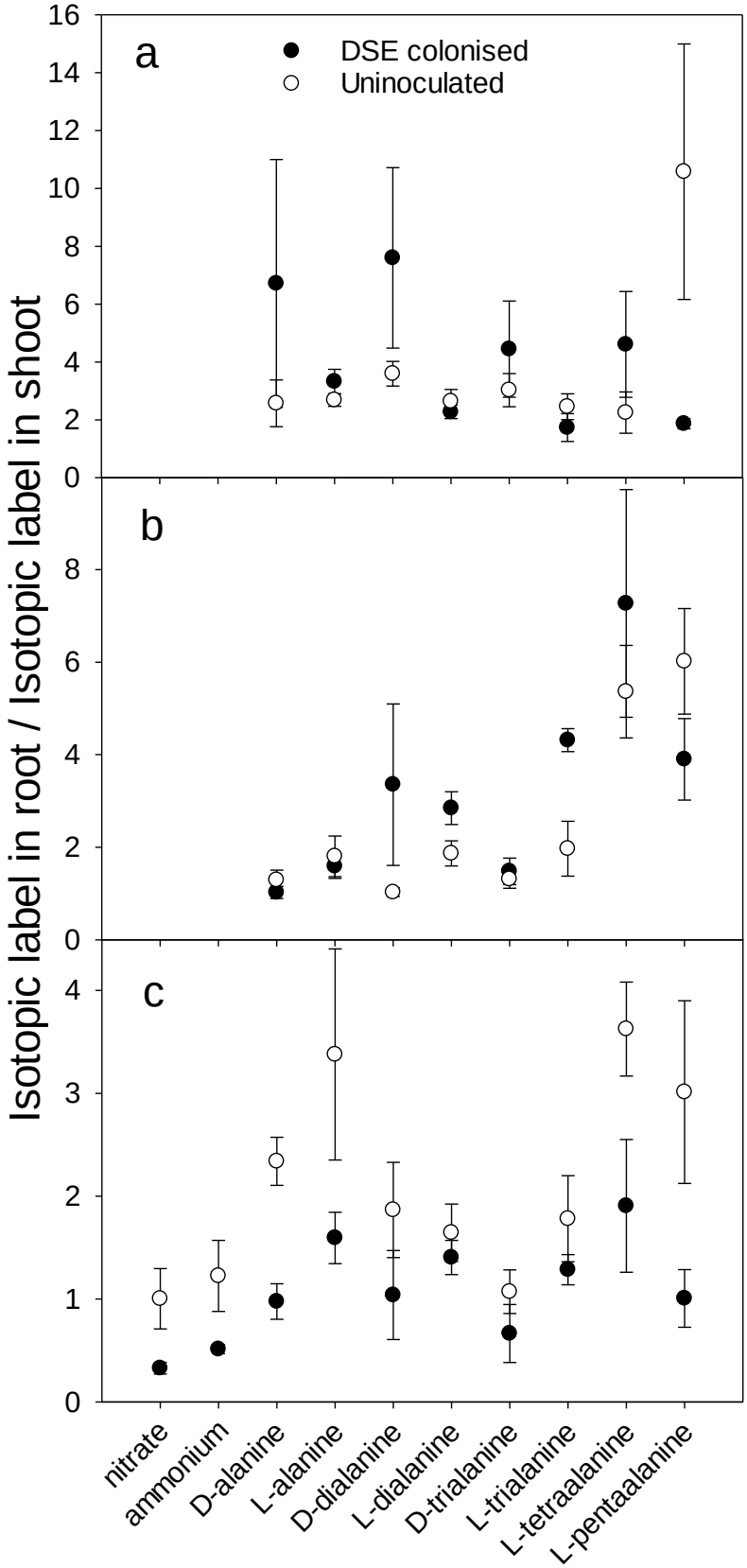
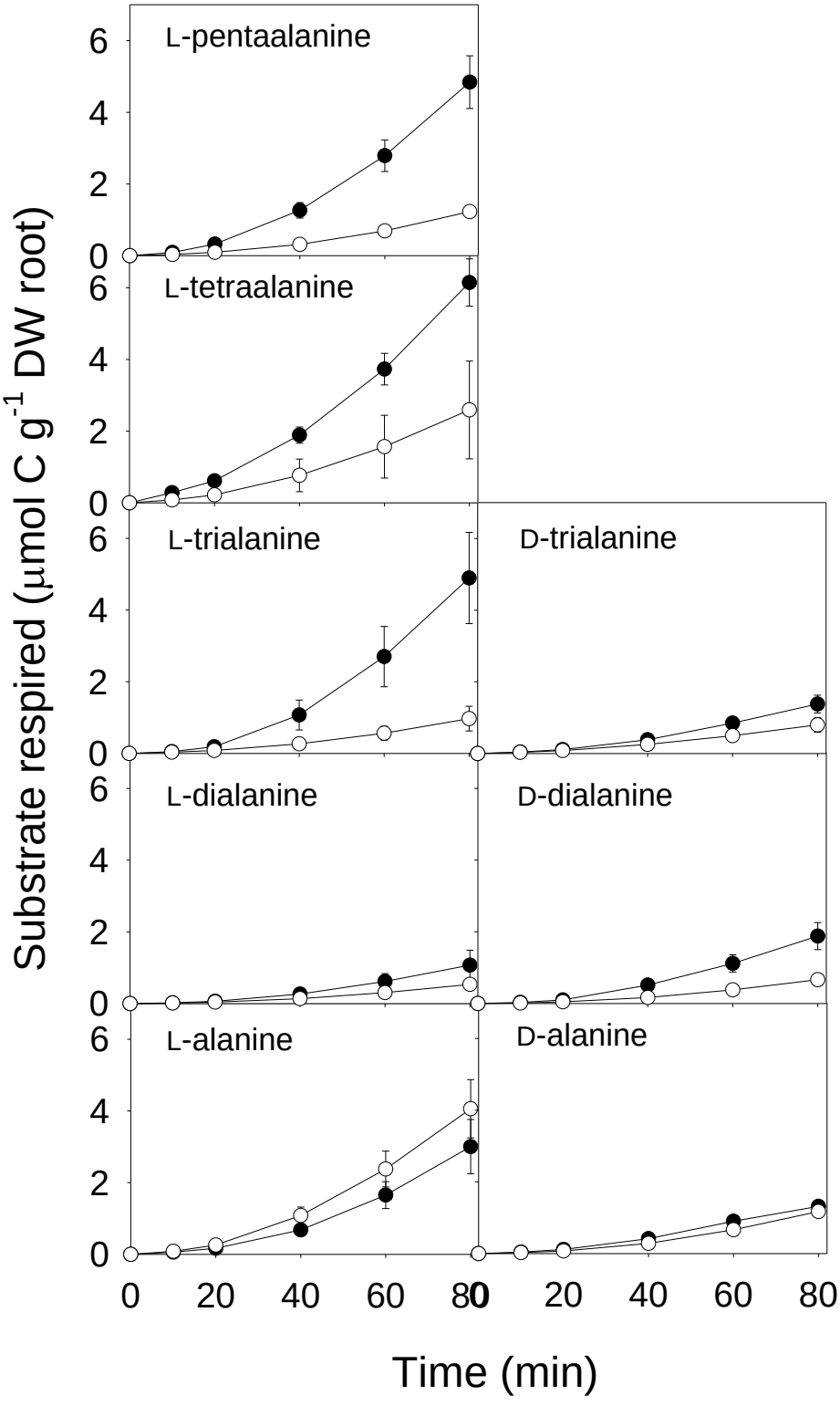


Figure S6 Ratio of recovery of isotopic labels in roots to recovery in shoots of *D. antarctica*. a. ^{14}C. b. ^{13}C. c. ^{15}N. Data are mean ± SEM; *n*=3 for ^{14}C; *n*=4 for ^{13}C and ^{15}N.



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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.

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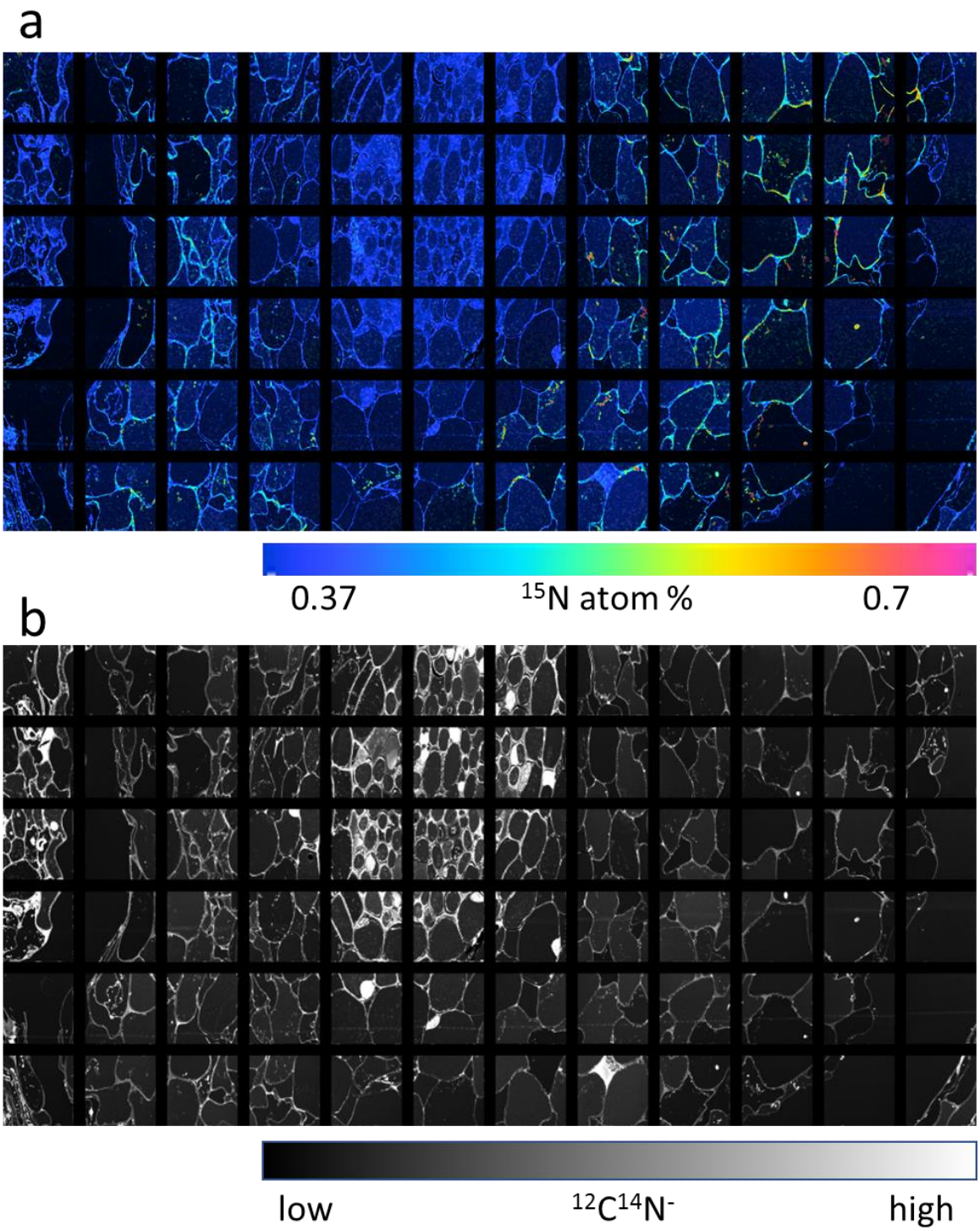

Figure S8 ^{15}N enrichment image of entire root cross-section of *D. antarctica* inoculated with DSE and incubated for 5 min in ^{15}N L-trialanine. Similar montaged images were generated for all four treatments (+/-DSE, D or L ^{15}N-trialinine) and an unlabelled control. For the example displayed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

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