



Rethinking the sub-Antarctic terrestrial N-cycle: evidence for organic N acquisition by Marion Island grasses

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Abstract

Organic N (oN, e.g., amino acids) is an important N-resource for plants in soils replete with oN but not inorganic N (iN; i.e., NH_4^+ and NO_3^-), such as cold ecosystems with temperature-limited soil decomposition rates. However, sub-Antarctic literature assumes that plants only acquire iN, potentially underestimating plant-available N. We hypothesised that Marion Island (-46.90° , 37.75°) grasses (*Polypogon magellanicus*, *Poa cookii*, *Agrostis stolonifera* and *Poa annua*) acquire oN and that oN relative to iN provision affects plant growth. We investigated oN and iN uptake and growth responses in two hydroponics experiments. In situ N (^{15}N -glycine, $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$) acquisition was investigated at three field sites with decreasing faunal influence, thus iN input and microbial activity. When plants grown in mire water were supplied with ^{15}N -glycine or $^{15}\text{NO}_3^-$, root $\delta^{15}\text{N}$ enrichment was highest for glycine-supplied plants. In the second hydroponics experiment, plant N-uptake rates ($\text{nmol g biomass}^{-1} \text{ s}^{-1}$) were significantly higher for glycine than NO_3^- , but relative growth rates ($\text{g g}^{-1} \text{ d}^{-1}$) lower on glycine. There were species-specific biomass allocation responses to N concentration (4 mM and 0.4 mM) and N-form (glycine and NO_3^-). Glycine-supplied grasses at the low iN concentration field sites had significantly higher $\delta^{15}\text{N}$ enrichment relative to those at sites with high iN, suggesting higher oN uptake when iN is limiting. We demonstrate the importance of accounting for oN acquisition in the sub-Antarctic. As a system with high soil oN relative to iN, plants may predominantly meet N-demands through oN rather than iN acquisition.

Keywords Nitrogen · Graminoid · Sub-Antarctic · Amino acid · N-cycling · N-uptake · Organic N · Nutrient cycle

Introduction

Plant productivity is a key component of the terrestrial N-cycle, and thus identifying plant-available N sources substantially influences our understanding of it. Until the late twentieth century, the soil N-cycle was understood to be regulated by microbial mineralisation of soil organic matter (SOM) (Schimel and Bennett 2004). Most literature assumed inorganic N (iN; i.e., NH_4^+ and NO_3^-) was the only N source ecologically relevant for plant acquisition (Smith and Steenkamp 1992a), despite the capacity for direct organic N (oN,

e.g., free amino acids) acquisition being long established (e.g., Hutchinson & Miller 1912; Virtanen & Linkola 1946; Wright 1962). However, instead of microbial mineralisation, the soil N-cycle is largely driven by the depolymerisation of SOM by extracellular enzymes, which release oN monomers and polymers for acquisition by both plants and microbes (Schimel and Bennett 2004). This challenges two important assumptions in N-cycling literature. First, that microbes are always superior competitors for oN compared to plants, with evidence for successful in situ plant competition for oN (Lipson and Monson 1998; Hodge et al. 2000; Schmidt et al. 2002). Secondly, and the focus of this paper, that plants rely only on iN pools to meet their N-requirements.

Plants can acquire oN molecules both directly, mediated by root transporters, or through mycorrhizal symbionts (Miller and Cramer 2004; Näsholm et al. 2009; Acuña-Rodríguez et al. 2020). There is widespread evidence for plant oN acquisition, spanning a range of ecosystems. For example, oN acquisition has been documented in Arctic tundra (Kielland 1994, 2001; Nordin et al. 2004), the Antarctic

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(Hill et al. 2019), temperate grasslands (Streeter et al. 2000; Bardgett et al. 2003), as well as boreal, temperate, and tropical forests (Nordin et al. 2001; Finzi and Berthrong 2005; Andersen and Turner 2013). However, it is generally accepted that oN acquisition is most prevalent—and indeed necessary for plant N budgets—in soils replete in oN and not iN (Weigelt et al. 2003; Jones et al. 2005b). This is especially relevant in cold ecosystems, where bioavailable soil N is regulated by low temperatures limiting extracellular enzyme activity and microbial mineralisation, effectively resulting in higher bioavailable oN than iN concentrations (Kielland 1994, 2001; Atkin 1996). Indeed, iN availability in Arctic and sub-Arctic ecosystems is insufficient to meet plant requirements without the direct uptake of oN (Kielland 2001; Lipson and Näsholm 2001; Näsholm et al. 2009). Therefore, investigations into cold systems that only considered plant iN uptake may have greatly underestimated plant-available N. This will have important implications for describing and quantifying plant-soil-N fluxes.

Despite a wealth of literature on plant oN uptake and its importance for N budgets in northern high latitudes, oN acquisition by sub-Antarctic flora has been largely overlooked, (with one exception; Schmidt and Stewart 1999). Furthermore, most sub-Antarctic nutrient- and/or N-cycling descriptions (e.g., Bergstrom and Chown 1999; Pendlebury and Barnes-Keoghan 2007; Selkirk 2007) pivots on work that assumed plant-available N was limited to iN, without accounting for potential oN acquisition (Smith and Steenkamp 1992a). The vegetated lowland (below 300 m a.s.l) soils of sub-Antarctic islands, such as Marion Island (− 46.90°, 37.75°), are typically highly organic (Smith 1978a). For example, soil oN on Marion Island makes up ca. 99.9% of total soil N (20 mg g^{−1} oN and < 0.1 mg g^{−1} iN, Table S1) if uninfluenced by large vertebrates such as seabirds and pinnipeds (Smith 1978b). Furthermore, plant N-requirements (158 mg N m^{−2} d^{−1}; Smith 1988) of a Marion Island mire-grassland community during the growing season are not met by the low iN mineralisation rates (net mineralisation 48 mg N m^{−2} d^{−1}) (Smith and Steenkamp 1992a). These authors assumed that only iN was relevant for plant nutrition and iN excretion by soil macrofauna was assumed to account for this discrepancy (Smith and Steenkamp 1992b, c, 1993; Smith 2008). Measurements of dissolved organic N (DON), which more accurately represent bioavailable N at a given time, show higher concentrations of DON than iN at sites with lower vertebrate influence (e.g., 82% of total dissolved N, TDN at a coastal site), and similar DON and iN concentrations when proximal to vertebrates (e.g., 58% of TDN is oN; Table S2). However, to date there has been no investigation into whether plants in this ecosystem are able to access the large pool of oN.

In addition to the important role oN acquisition may play in plant nutrition, there is evidence that oN relative to iN uptake affects plant growth and allocation. Compared to iN, the simultaneous uptake of C with N provides a net assimilation advantage to oN acquisition, the so-called “C-bonus” (Zerihun et al. 1998; Franklin et al. 2017). For example, oN (6 mM glutamine) relative to equimolar iN (NO₃[−] and NH₄NO₃) increases *Arabidopsis thaliana* relative growth rates and root fractions, due to higher N use efficiency (NUE) and the low diffusibility of oN compounds in soil, respectively (Cambui et al. 2011; Franklin et al. 2017). However, plant responses to N-forms differ, as studies have also shown decreased root investment with amino acid application (Walch-Liu et al. 2006; Miller et al. 2007). These differences may be species-specific or depend on the concentration of the respective N-forms provided (Walch-Liu et al. 2006; Lonhienne et al. 2014). However, should N-form affect plant nutrition and allocation in the natural environment, the N-form predominantly acquired may have important ecological implications. While the effects of N-form and N concentration have been widely studied, most of this literature focusses on agricultural species (e.g., Padgett and Leonard 1996; Jämtgård et al. 2008; Tian et al. 2008; Hassan et al. 2020) or the model plant *A. thaliana* (Cambui et al. 2011; Lonhienne et al. 2014; Kiba and Krapp 2016). With less literature investigating how N-forms or N concentration affect wild plant growth, little is known of the ecological significance of oN or iN uptake, such as its effects on plant growth traits, competition, or co-existence in situ.

This study investigated oN uptake and the effects of oN vs iN provision on plant growth in four Marion Island graminoid species, *Polypogon magellanicus* (Lam.) Finot (previously *Agrostis magellanica*), *Poa cookii* (Hook.f.) Hook.f., *Agrostis stolonifera* L. and *Poa annua* L. We hypothesised that Marion Island grasses have the capacity for oN uptake, that oN influences RGR and biomass partitioning, and that the grasses acquire oN in situ, predicting that oN acquisition would be highest in soils with low faunal influence and low iN. The capacity for potted and in situ oN and iN uptake was determined by providing plants with ¹⁵N labelled oN (glycine) or iN (¹⁵NO₃[−] and ¹⁵NH₄⁺). The effects of oN (glycine) and iN (NO₃[−]) on plant growth and biomass partitioning were explored in a hydroponics experiment.

Materials and methods

Study site and species

Sub-Antarctic terrestrial biotas are confined to several small, volcanic islands in the Southern Ocean (Selkirk 2007). The islands are characterised by a hyperoceanic climate, with

limited seasonal range of cool temperatures. Sub-Antarctic Marion Island experiences a mean annual temperature of 6 °C, with a *ca.* 4 °C seasonal range between mean winter and summer temperatures (le Roux 2008). Soils near the coast and up to *ca.* 300 m a.s.l. are highly organic as they hold large amounts of plant material at various stages of the decomposition process. The rates of microbial decomposition of this material is slow, limited by a combination of the low temperatures and excessive moisture contents of the soils (Smith 1987). In comparison to the reported net iN mineralisation rates of 48 mg m⁻² d⁻¹ (Smith and Steenkamp 1992a), iN mineralisation rates in another cold system (an Arctic sedge meadow) is 123.4 mg m⁻² d⁻¹ (Chapin 1996). Furthermore, and assuming a root depth of 30 cm, Marion Island mineralisation rates are 0.26 mg kg⁻¹ d⁻¹, which is near the lowest rates reported in a global synthesis from Risch et al. (2019). Decomposition and iN release by invertebrates is limited due to current high predation by the invasive house mouse (Smith 2002; Smith et al. 2002; McClelland et al. 2018).

The four grasses investigated included two native species, *P. magellanicus* and *P. cookii*, and two invasive (anthropogenically introduced) species, *P. annua* and *A. stolonifera*. *Poa cookii* is a tussock-forming, highly nitrophilous species occurring in areas influenced by vertebrates (such as seabirds and pinnipeds) (Smith 1976a). *Polypogon magellanicus* is a tussock-forming species with a widespread distribution across Marion Island, as a common coastal and mire species but also found inland growing epiphytically on *Azorella selago* cushions (Smith et al. 2001). *Agrostis stolonifera* is a stoloniferous, invasive species that was first recorded in 1965 and has since had major impact on the island, having spread along the coastline and where there has been human activity (le Roux et al. 2013; Greve et al. 2017). It is native to parts of Europe, Asia, and north Africa, but invasive on all other continents except for Antarctica (Govaerts et al. 2021). *Poa annua*, another stoloniferous and invasive species, was first recorded on Marion Island in 1948 but was probably introduced by sealers in the 1800s and has also spread along the coastline (le Roux et al. 2013; Greve et al. 2017). It is native to parts of Africa, Europe, and Asia, but is invasive on all other continents including Antarctica (Molina-Montenegro et al. 2019; Govaerts et al. 2021). All four grasses co-occur along sections of the Marion Island coastline.

Two hydroponics experiments were conducted. In the first hydroponics experiment, grasses were each grown in 1 L of water that was collected from a large mire on the east coast of the island (− 46.876°, 37.857°). Mires cover *ca.* 50% of the island below 300 m a.s.l. and thus contribute to a substantial fraction of the island's annual productivity (Smith 1988; Smith et al. 2001). The experiment was conducted outdoors under natural light and temperature near

the main base during March 2020. The second hydroponics experiment was conducted in South Africa at the University of Cape Town. The grasses were collected from the same site as the former experiment, and grown in 1 L Long Ashton (LA) nutrient solution (Hewitt 1966).

The field experiment investigated in situ uptake of glycine, NO₃⁻, and NH₄⁺ at three sites (Table S) on the east coast of Marion Island. The sites had varying degrees of vertebrate influence (thus allochthonous iN input) and were characterised according to the descriptions in Smith et al. (2001) as 'Biotic' for the site with high animal influence near the beach, 'Slope' for the site along a fern slope-complex on black lava, and 'Fellfield' for the site on a grey-lava ridge (Fig. 1; Table S4). All four species were present within 30 m of each other at the 'Biotic' site, which had high vertebrate influence by seabirds such as *Diomedea exulans* (Wandering Albatross); *Aptenodytes patagonicus* (King Penguins), and *Eudyptes chrysocome* (Southern Rock Hopper Penguins) and pinnipeds *Mirounga leonina* (Southern Elephant Seals) and *Arctocephalus gazella* (Antarctic Fur Seals). This site was mostly covered by the four grasses, bryophytes, and the herb *Leptinella plumosa*. The 'Slope' site, with only *P. cookii* present, had some inactive Procellariidae (petrels and prions) burrows, representing a site with intermediate vertebrate influence (Smith 1976a). The slope was dominated by the fern, *Austroblechnum penna-marina*, with some *P. cookii* tussocks. The 'Fellfield' site had no visible vertebrate influence, although it may have been frequented by mice. This site was sparsely vegetated, with some *Azorella selago* cushions, *Andreaea* spp. and *Ditrichum* spp. cushion bryophytes, *P. magellanicus* and *P. annua* individuals. Seabirds and pinnipeds fertilise the soil with both oN and iN compounds (Wu et al. 2023). The soil iN, broken down from uric acid and urea, are dominated by NH₄⁺, which is either washed out to sea, undergoes nitrification, or volatilised to NH₃ and carried away by wind (Smith 1978b; Erskine et al. 1998; Wu et al. 2023).

Hydroponics experiments: uptake of and plant growth on organic and inorganic N

To determine whether sub-Antarctic grasses have the capacity for oN uptake, mature, non-flowering tillers of each species (*n* = 12) were grown for five weeks at the end of the growing season (March–April 2020). Each replicate was grown in 1 L mire water (see Table S5) with 6 mM NaNO₃, with water change and nutrient replenishments every two weeks. At five weeks, plants (*n* = 6 of each species) were supplied with either 6 mM ¹⁵N-glycine (98% ¹⁵N, Cambridge Isotope Laboratories, Andover, MA, USA) or ¹⁵N-NaNO₃ (98% ¹⁵N, Cambridge Isotope Laboratories). The ¹⁵N solutions consisted of 20 mL 10.5 μM glycine and 20 mL 10.5 μM NO₃⁻, one of which was isotopically distinct

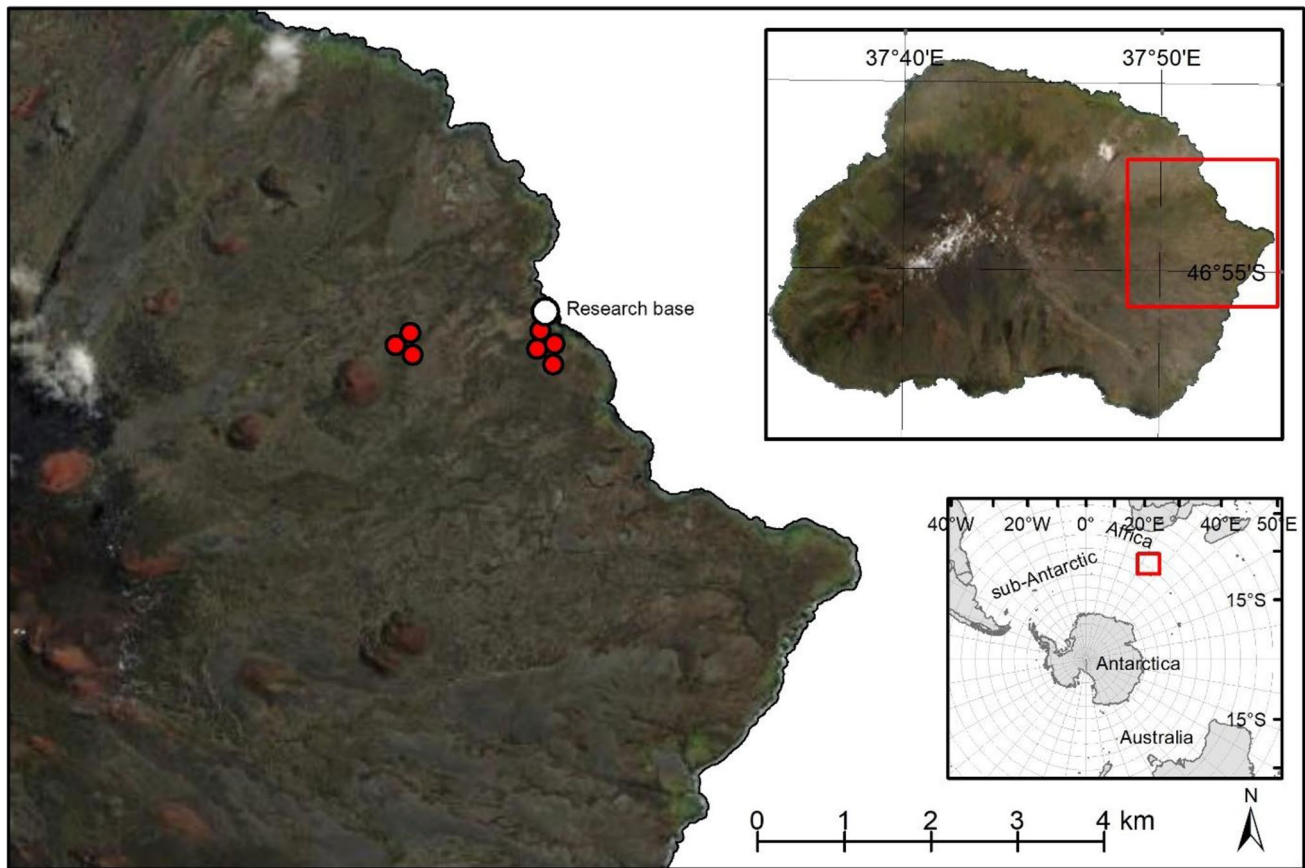


Fig. 1 Map of sub-Antarctic Marion Island. The white point shows the research base on the east coast, and the red points show the locations where ^{15}N enriched oN and iN were provided to the four grasses. The points near the research base are for *Polypogon magellanicus*, *Poa cookii*, *Poa annua* and *Agrostis stolonifera* and the

points further inland near the base of a scoria cone (known on the island as “Junior’s Kop”) are for *Polypogon magellanicus* and *Poa annua* on a grey-lava outcrop and *Poa cookii* on a slope ca. 150 m from the grey-lava

(i.e., contained ^{15}N). One leaf was harvested at 12 h, and the remainder of the plant harvested at 24 h following ^{15}N supply. Unenriched (natural abundance) leaf $\delta^{15}\text{N}$ values were obtained from plants in a separate experiment with the same growing medium.

The LA hydroponics experiment investigated plant growth, biomass partitioning, and N-uptake rates on different concentrations (4 mM or 0.4 mM) and forms of N (glycine or NO_3^-). Plants ($n = 6$ of each species) were grown in LA solution (4 mM CaCl_2 , 2 mM K_2SO_4 , 1.1 mM MgSO_4 , 0.14 mM H_3BO_3 , 90 μM Fe-EDTA, 20.8 μM MnSO_4 , 3.3 μM CuSO_4 , 2.3 μM ZnSO_4 , 0.25 μM Na_2MoO_4 , and a phosphate buffer (pH 5.5) of 1.87 mM NaH_2PO_4 and 0.13 mM Na_2HPO_4), modified from Hewitt (1966). This experiment was run in a controlled growth chamber with a 12/12 light–dark cycle, and PAR ca. 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at 10 °C. A pilot experiment showed that 4 mM glycine in LA solution formed a precipitate within 12 h due to changes in solution pH. To avoid this, an asynchronous design was adopted, where plants

were transferred between 1 L LA solution and 1 L 2 mM $\text{CaCl}_2 + 4/0.4$ mM-N every 24 h, maximising time for nutrient acquisition but minimising movement and thus root damage. Every eight days, nutrients were replenished, and fresh biomass weighed to determine plant RGR according to the methods described by Hoffmann and Poorter (2002). At 40 d, plants were left in their respective N treatments under constant light and 1 mL solution was sampled every 12 h for 48 h, to determine N-uptake rates. To account for water loss through evapotranspiration, initial and final solution volumes were incorporated into uptake rate calculations. Plants were then harvested, dried, and weighed.

The second fully expanded leaf from the apical meristem from each plant was used for $\delta^{15}\text{N}$ (‰) and leaf N (%) analyses. Harvested material was dried for 48 h at 70 °C, and the dry weights used to calculate root:shoot ratios. The main tiller of two replicates of *P. cookii* in the 4 mM NO_3^- treatment died during the experiment, which caused excessive variation in root:shoot ratio due

to the low mass of the emerging tillers, and thus these two replicates were excluded from root:shoot ratio analysis.

Organic and inorganic N uptake in situ

To determine whether plants access oN in situ and whether this was influenced by nutrient inputs by vertebrate influence, plants were provided with ^{15}N -glycine, $^{15}\text{N}\text{-NO}_3^-$, or $^{15}\text{N}\text{-NH}_4^+$ ($^{15}\text{NH}_4\text{Cl}$, 98% ^{15}N , Cambridge Isotope Laboratories, Andover, Ma, USA). One leaf ($n = 10$ of each species at each site) was sampled 24 h before enrichment to determine unenriched $\delta^{15}\text{N}$ (i.e., natural $\delta^{15}\text{N}$ abundances). ^{15}N treatments were added to plants ($n = 10$) at each site between 8 and 10 am, and one leaf harvested 24 h later. Each labelling solution (60 mL) comprised 20 mL 10 μM glycine, 20 mL 10 μM NO_3^- and 20 mL 10 μM NH_4^+ , one of which was isotopically distinct (i.e., contained ^{15}N). All leaves harvested for $\delta^{15}\text{N}$ analyses were dried for 48 h at 70 °C and stored at room temperature until $\delta^{15}\text{N}$ analyses.

Colorimetric assays of glycine and NO_3^-

Colorimetric assays were used to determine glycine and NO_3^- concentrations in the LA growth experiment nutrient solution, to calculate N-uptake rates. NO_3^- was measured using the method described by Doane and Horwath (2003), where VCl_3 reduces NO_3^- to NO_2^- , which is captured by Griess reagents (N-1-naphthylethylenediamine dihydrochloride and sulphanilamide). Glycine was measured according to Moore and Stein (1954), where NH_2 -containing groups are reacted with a ninhydrin reagent. In samples without glycine addition, there were negligible amounts of NH_2 -containing groups, and therefore no correction was carried out for the ninhydrin protocol. Absorbance of colorimetric reactions was measured with a ThermoSpectronic spectrophotometer (Helios Epsilon model, Thermo Scientific, USA) at 540 and 570 nm for NO_3^- and glycine, respectively.

Leaf $\delta^{15}\text{N}$ and leaf N analyses

For $\delta^{15}\text{N}$ and leaf N analyses, plant leaves were ground to a fine powder using a ball mill (MM200, Retsch, Germany), and 2 mg of each sample weighed into tin capsules. The tin capsules were combusted in a Flash 2000 organic elemental analyser (Thermo Scientific, Germany), and the gasses passed into a DELTA V Plus IRMS (isotope ratio mass spectrometer) via a ConFlo IV gas control unit. Results were calibrated according to in-house standards.

Statistical analyses

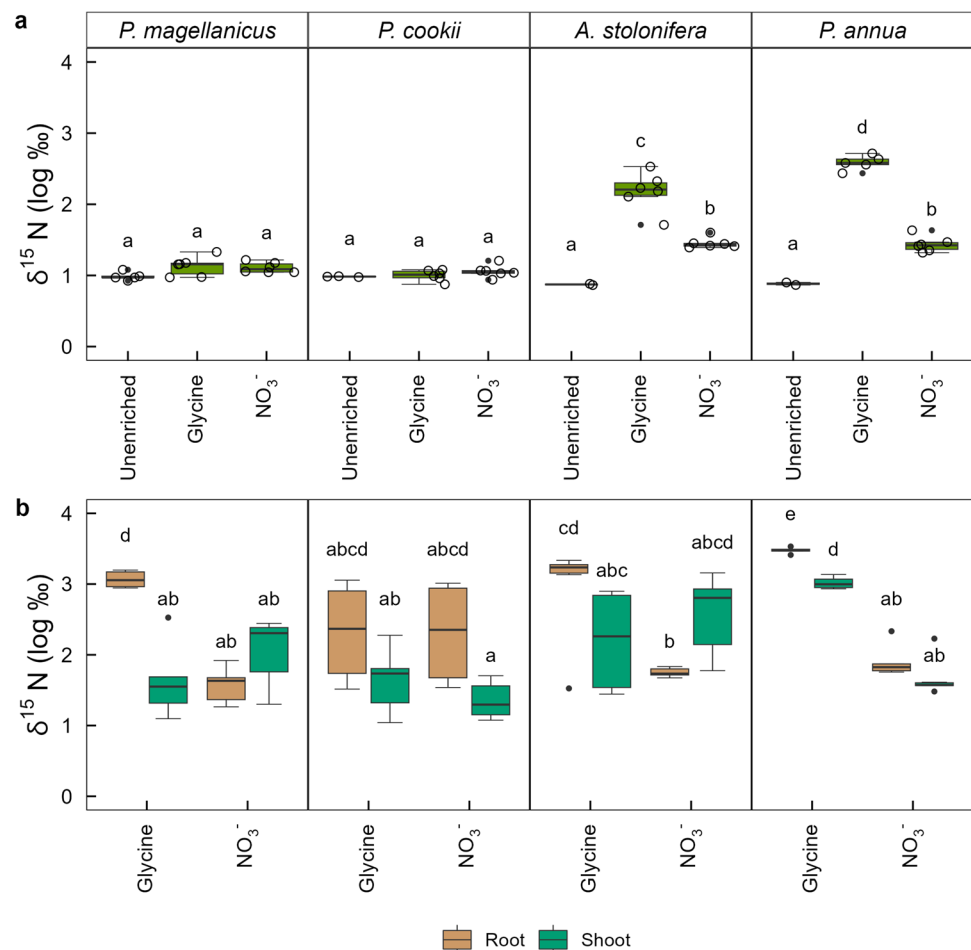
All statistical analyses were performed using R statistical software, version R.4.1.1 (R Core Team 2021). Data were analysed using an Analysis of Variance (ANOVA), testing for interacting effects between species and the respective fixed effects in each experiment (i.e., plant tissue or site and N-form in the ^{15}N enrichment experiments; N-form and -concentration for the LA growth experiment) on plant $\delta^{15}\text{N}$ and growth variables. All residuals were checked (by visually assessing residual distributions) to conform to model assumptions, and where they did not, the data were log-transformed to meet normality and homoscedasticity assumptions. For root and shoot $\delta^{15}\text{N}$, log transformations did not improve homoscedasticity, and so a model was fit with the generalised least-squares (GLS) method (Zuur et al. 2009) in the ‘nlme’ package (Pinheiro and Bates 2000). Where analyses revealed significant effects ($p < 0.05$), Tukey Honest Significant Differences post-hoc tests were performed in the ‘emmeans’ package (Lenth 2023). For the LA and field experiments, interactions in the three-way ANOVAs resulted in complicated outputs. Because these results indicated species-specific responses to the treatments, a separate ANOVA was used for each species separately to aid interpretation. To determine the specific uptake rates over 48 h in the LA growth experiment, linear models were fitted to the solution N concentration (glycine or NO_3^-) over time. Uptake rates were considered detectable if there was a significant relationship ($p < 0.05$) between time and N concentration, and the slopes from these models used to calculate uptake rates (nmol g^{-1} plant biomass s^{-1}). Uptake rates were then analysed with an ANOVA with N-treatment as factor. Where further investigations into *a-priori* hypotheses were relevant (i.e., for iN and oN uptake rates across all species in the LA experiment), contrasts were performed using the ‘emmeans’ package. All ANOVAs were performed in the ‘car’ package (Fox and Weisberg 2019).

Results

Hydroponics experiments: uptake of and growth on organic and inorganic N

All four grasses showed significant enrichment with both ^{15}N -glycine and $^{15}\text{N}\text{-NO}_3^-$ when it was supplied in hydroponics (Fig. 2). Leaf material (harvested at 12 h; Fig. 2a) of *A. stolonifera* and *P. annua* had significant ^{15}N enrichment with both N-forms, and significantly higher glycine-than NO_3^- -enrichment. Neither *P. magellanicus* nor *P. cookii* showed significant enrichment in the leaf material. Overall, when compared to the unenriched leaf $\delta^{15}\text{N}$ ($9 \pm 0.4\%$ mean \pm SE), root and shoot $\delta^{15}\text{N}$ increased substantially

Fig. 2 $\delta^{15}\text{N}$ of the four study species (*Polypogon magellanicus*, *Poa cookii*, *Agrostis stolonifera* and *Poa annua*) supplied with ^{15}N -glycine or ^{15}N - NO_3^- . **a** $\delta^{15}\text{N}$ 12 h after ^{15}N enrichment in either unenriched leaves, or leaves supplied with ^{15}N -glycine or ^{15}N - NO_3^- . There was a significant interaction between species and N-treatment ($F_{6,47} = 53.04$, $p < 0.0001$). Points are included to indicate the replication. **b** $\delta^{15}\text{N}$ 24 h after ^{15}N enrichment with either ^{15}N -glycine or ^{15}N - NO_3^- . There was a significant interaction between species, plant tissue, and N-form ($F_{3,79} = 13.76$, $p < 0.0001$). Data are plotted on a log scale to aid visual interpretation. Letters indicate significant differences between treatment and species from an emmeans post-hoc test at the $\alpha = 0.05$ significance level. Green boxplots show the shoot and leaf $\delta^{15}\text{N}$, and brown boxplots the root $\delta^{15}\text{N}$

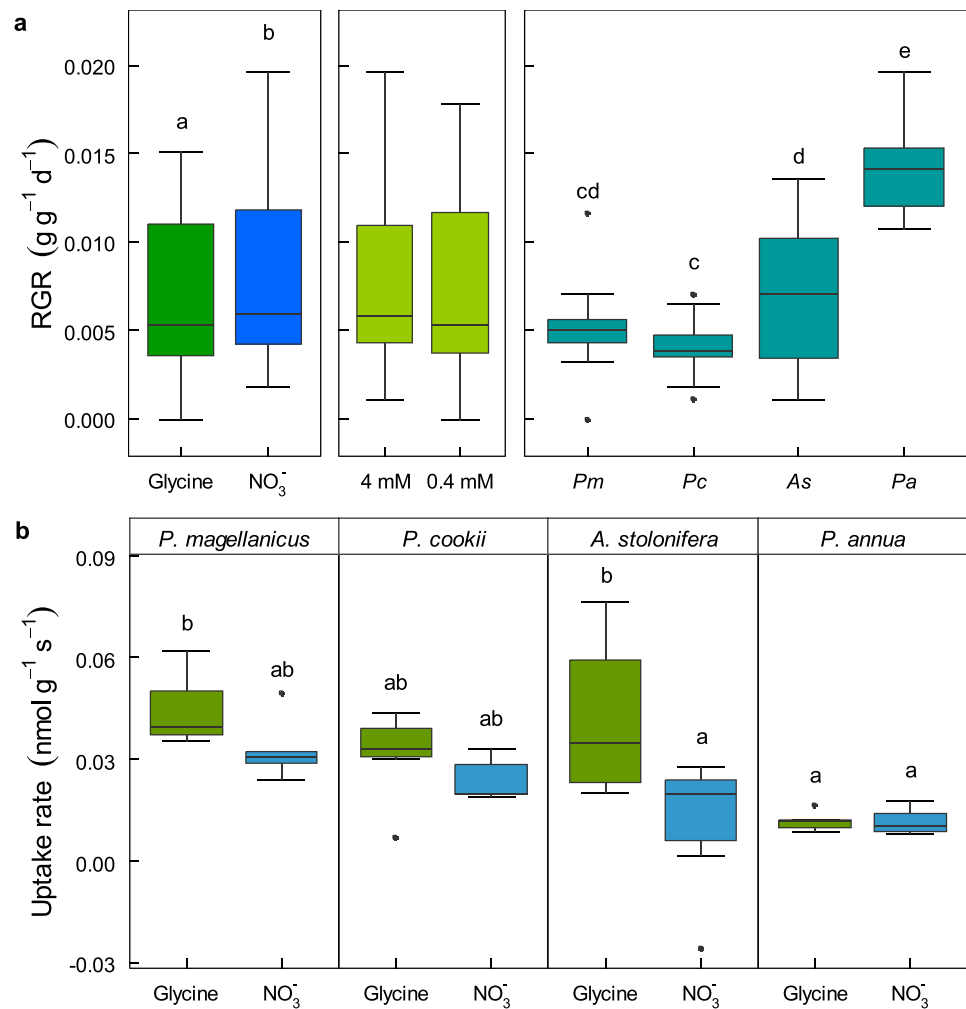


(mean $\delta^{15}\text{N} > 100\%$ for glycine and NO_3^-) following ^{15}N provision (Fig. 2a, b). Furthermore, root $\delta^{15}\text{N}$ was significantly higher following glycine than NO_3^- provision for all species except *P. cookii* (Fig. 2b). There was a significant effect of species, N-form, and plant tissue in root and shoot $\delta^{15}\text{N}$ ($p < 0.05$). The best fit gls model (with the lowest AIC value) accounted for the variance structure of all three explanatory variables.

In the LA growth experiment, there was no significant interaction between treatments and species for plant RGR. However, RGR was significantly higher in the NO_3^- than glycine treatment, and *P. annua* had significantly higher RGR than the other three species (Fig. 3a). Only *P. annua* resulted in a detectable decrease in N concentration in the nutrient solutions of the 4 mM-N treatments ($p < 0.05$; Fig. S1), and so uptake rates were analysed and compared across species in the 0.4 mM treatments only. There was a significant interaction between species and N-treatment ($p < 0.05$; Fig. 3b) for N-uptake rates, with higher glycine than NO_3^- uptake rates by *A. stolonifera*. Glycine uptake (nmol g^{-1} plant biomass s^{-1}) was higher than the uptake of NO_3^- across all species (contrast: $t = 3.28$, $p = 0.0022$; Fig. 3b).

There was a significant interaction between treatments and species in the LA growth experiment for root:shoot ratios and leaf N (Fig. 4), showing species-specific responses to N-form (root:shoot: $F_{3,78} = 5.86$, $p = 0.0011$) and N concentration (root:shoot: $F_{3,78} = 4.62$, $p = 0.0050$; leaf N: $F_{3,79} = 27.0$, $p < 0.0001$). When each species was analysed separately, there was evidence that N-form (glycine relative to NO_3^-) had a strong effect on *P. magellanicus* irrespective of (total) N concentration (4 mM relative to 0.4 mM). Here, root:shoot ratios were significantly lower on glycine than NO_3^- . By contrast, N concentration had a strong effect on *P. annua* root:shoot ratio and leaf N irrespective of N-form, with high root proliferation and low leaf N at 0.4 mM relative to low root growth and high leaf N at 4 mM (Fig. 4). Both N concentration and N-form had an interactive effect on *P. cookii* root:shoot ratio and leaf N, which were both highest at 4 mM NO_3^- but lowest at 4 mM glycine. While neither N concentration nor N-form affected *A. stolonifera* root:shoot ratio, they had an interactive effect on leaf N, which increased with N concentration but significantly more so with NO_3^- than glycine (Fig. 4a, b).

Fig. 3 Growth in the four N treatments and uptake rates in the two 0.4 mM treatments for all study species (“Pm” is *Polypogon magellanicus*, “Pc” is *Poa cookii*, “As” is *Agrostis stolonifera*, and “Pa” is *Poa annua*) in the LA experiment. **a** Relative growth rate (RGR), where there was a significant difference in RGR between the N-form ($F_{1,90}=5.87$, $p=0.0174$; left panel) and between the four species ($F_{3,90}=80.58$, $p<0.0001$; right panel), but no difference between N concentration (middle panel). The high overlap between the boxes in the left panel is due to the interspecific variation in the different species’ RGR. **b** N-uptake rate ($\text{nmol g}^{-1} \text{s}^{-1}$), where there was a significant interaction between N-form (NO_3^- or glycine) and species ($F_{3,40}=2.92$, $p=0.0456$). Letters indicate significant differences between treatments and species, from an emmeans post-hoc test at the $\alpha=0.05$ significance level



Organic and inorganic N uptake in situ

The grasses showed significant $\delta^{15}\text{N}$ enrichment relative to controls in the field sites indicating uptake of the ^{15}N supplied. However, enrichment following oN or iN provision differed between species and sites, as shown by a significant interaction between these factors. There was significant $\delta^{15}\text{N}$ enrichment following glycine provision at the ‘Biotic’ (*A. stolonifera*) and ‘Fellfield’ (*P. magellanicus* and *P. annua*) sites but not the ‘Slope’ site (Fig. 5). By contrast, $\delta^{15}\text{N}$ enrichment following iN provision (NH_4^+ , NO_3^- or both) was documented at all sites for all species (Fig. 5). For the species present at multiple sites, site significantly affected $\delta^{15}\text{N}$ enrichment with the various N-forms. While there was only evidence for $\delta^{15}\text{N}$ enrichment in NO_3^- -provided *P. magellanicus* and *P. annua* at the ‘Biotic’ site, both species showed significant $\delta^{15}\text{N}$ enrichment in glycine- and iN-provided plants in the ‘Fellfield’ (Fig. 5). *Agrostis stolonifera*, which was only present at the biotic site, had significant enrichment with all ^{15}N treatments. *Poa cookii*, on the other

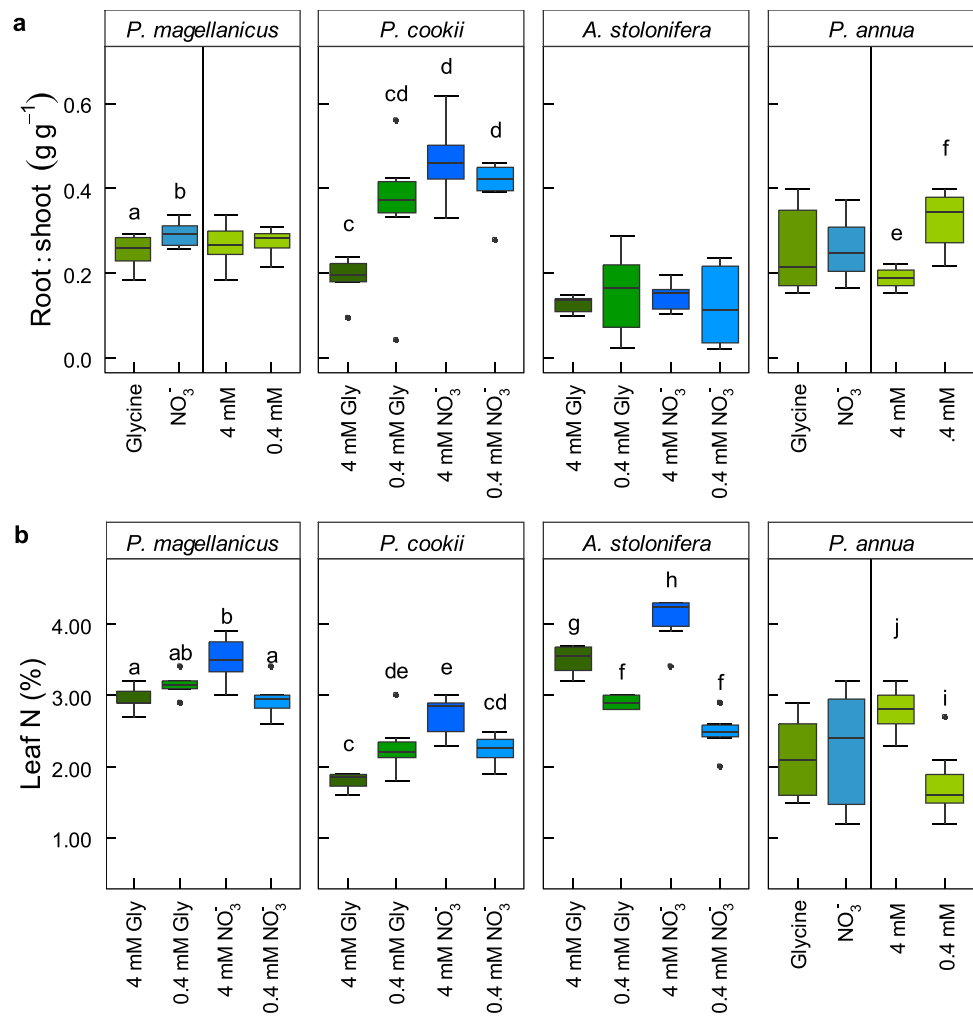
hand, only showed $\delta^{15}\text{N}$ enrichment with iN at both the ‘Biotic’ site (NO_3^-) and ‘Slope’ site (both iN forms) (Fig. 5).

Discussion

Sub-Antarctic grasses acquire oN, which is consistent with the literature showing that plants generally have the physiological capacity for oN uptake (Näsholm et al. 2009; Paungfoo-Lonhienne et al. 2012). Indeed, glycine uptake rates surpassed those of NO_3^- , both when measured over time and following ^{15}N provision. This indicates that the grasses have a high capacity for oN acquisition and readily acquire it when available at high concentrations. Considering the high oN concentrations and low mineralisation rates of Marion Island soils (Smith 1978b; Smith and Steenkamp 1992a), this suggests that direct oN acquisition plays an important, but overlooked, role in sub-Antarctic plant N nutrition.

The current study shows that sub-Antarctic grasses acquire oN in the field, particularly in sites with low iN.

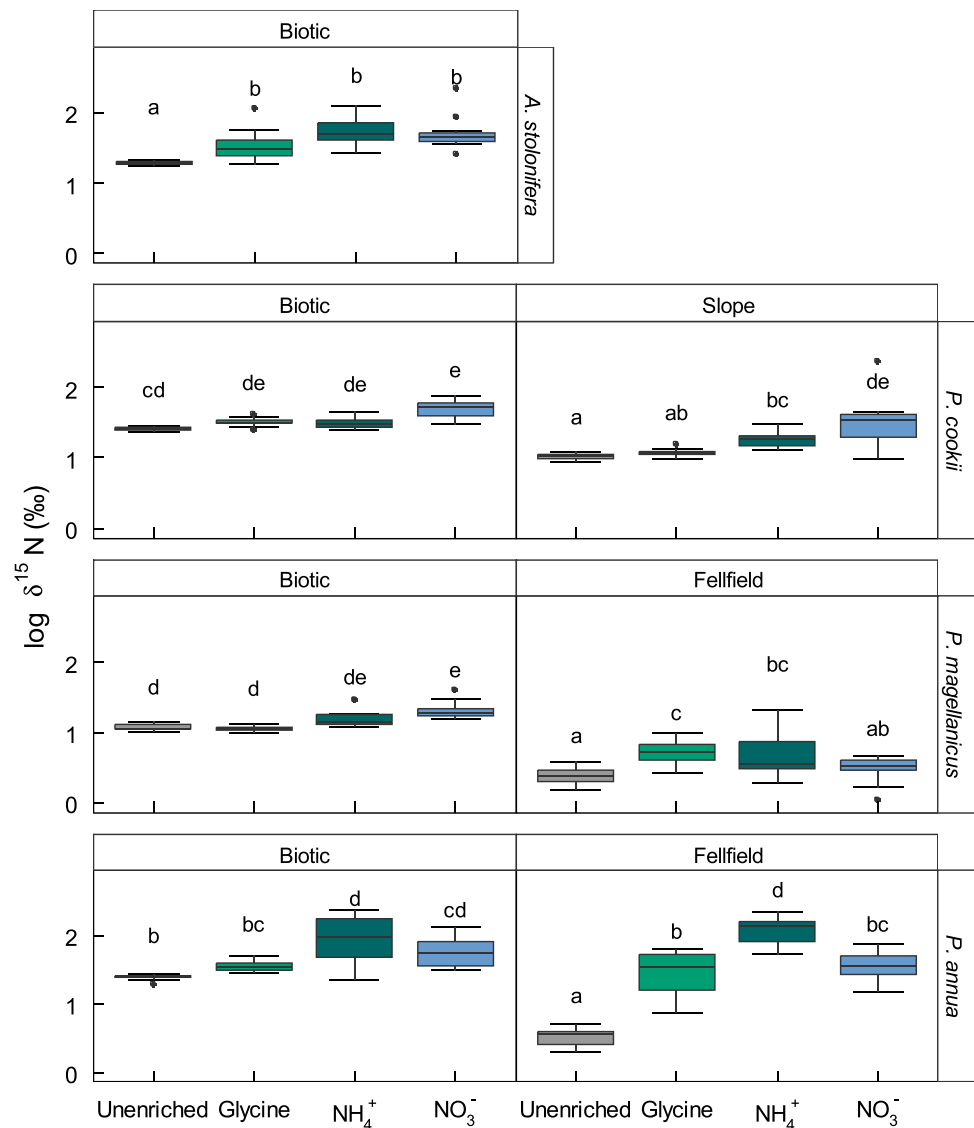
Fig. 4 Root:shoot ratio and leaf N for *Polypogon magellanicus*, *Poa cookii*, *Agrostis stolonifera*, and *Poa annua* the study species in the four N treatments in the LA growth experiment. **a** Root:shoot ratio, with a significant effect of N-form for *P. magellanicus* ($F_{1,21}=9.72$, $p=0.0052$) but no effect of N concentration; a significant effect of N concentration for *P. annua* ($F_{1,21}=46.60$, $p<0.0001$) but no effect of N-form; and a significant interaction between N concentration and N-form for *P. cookii* ($F_{1,18}=5.38$, $p=0.0323$). **b** Leaf N, where there was a significant interaction between N concentration and N-form for *P. magellanicus* ($F_{1,20}=14.00$, $p=0.0013$), *A. stolonifera* ($F_{1,19}=19.56$, $p=0.0003$) and *P. cookii* ($F_{1,20}=18.09$, $p=0.0004$); and a significant effect of N concentration for *P. annua* ($F_{1,21}=67.29$, $p<0.0001$) but no effect of N-form. Letters indicate significant differences from an emmeans post-hoc test at the $\alpha=0.05$ significance level



Plant oN use generally increases along gradients of oN availability (Schimel and Bennett 2004; Jones et al. 2005b; Moe 2013). We showed higher $\delta^{15}\text{N}$ enrichment in glycine-provided plants at the inland site, with lower vertebrate influence and thus soil iN concentrations compared to the coastal site where high densities of seals and seabirds provide high N enrichment. Furthermore, most grasses showed significant enrichment following iN but not oN provision at sites proximal to vertebrate activity with high soil iN. Allochthonous N input by vertebrates strongly increases soil iN concentrations directly and indirectly due to the increased microbial abundance and activity associated with them (Smith 1978b, 2003; French and Smith 1986; Grobler et al. 1987). Vertebrate influence and thus high soil iN availability on the island are, however, highly localized, with vertebrate colonies largely restricted to coastal areas (Smith 1978b). For example, iN forms 0.1–0.03% of soil N in mires (Smith 1976b), 0.1% of soil N on black lava slopes without Procellariidae burrows (Smith 1976a), and 0.01% of grey-lava soil N (Smith et al. 2001).

Due to the use of only singly labelled isotopes (^{15}N), intact acquisition of oN cannot be established due to the possibility of oN mineralisation prior to ^{15}N uptake (Persson and Näsholm 2001; Jones et al. 2005a; Warren 2012). Other studies attempt to circumvent this issue with dual-labelled $^{13}\text{C}^{15}\text{N}$, where positive regressions between the two isotopes show intact oN acquisition (Persson and Näsholm 2001; Jones et al. 2005a; Warren 2012). However, this method does not remove the chances of oN transformations: ^{13}C and ^{15}N may be taken up separately, or ^{13}C may be respired immediately following uptake; circumstances that may both result in misinterpretation of the N-form utilised (Warren 2012). In addition to the field ^{15}N data, we show oN uptake in the hydroponics experiments, where microbial activity is negligible. Other studies also show intact oN uptake in hydroponics experiments (e.g., Arkoun et al. 2012; Xiaochuang et al. 2015; Mohammadipour and Souri 2019). We therefore conclude that at least a component of the uptake is intact glycine. In the field, the low and often insufficient rates of iN mineralisation (Smith and Steenkamp

Fig. 5 Leaf $\delta^{15}\text{N}$ in the grasses at three sites in situ, for unenriched plants and plants supplied with ^{15}N -glycine, $^{15}\text{N}\text{-NH}_4^+$, or $^{15}\text{N}\text{-NO}_3^-$. The ‘Biotic’ site is rich in soil N and proximal to vertebrate influence, the ‘Slope’ near inactive burrowing petrel burrows and thus with intermediate N concentrations, and the ‘Fellfield’ site uninfluenced by vertebrates and thus the least rich in N. There was evidence for a significant interaction between species, site, and N-form ($F_{3, 251} = 11.32$, $p < 0.0001$), thus separate models were run for each species. There was a significant effect of N-form on *Agrostis stolonifera* $\delta^{15}\text{N}$ ($F_{3, 36} = 9.80$, $p < 0.0001$), and a significant interaction between site and N-form for *Poa cookii* ($F_{3, 72} = 3.31$, $p = 0.0248$), *Polypogon magellanicus* ($F_{3, 71} = 7.99$, $p = 0.0001$) and *Poa annua* ($F_{3, 72} = 19.14$, $p < 0.0001$) $\delta^{15}\text{N}$. Data were plotted on a log scale to aid visual interpretation. Letters show significant differences between N-forms and sites for each species from an emmeans post-hoc test at the $\alpha = 0.05$ significance level



1992a) suggests that intact oN is acquired in situ, but this requires further investigation. To this end, Kranabetter et al. (2007) show that DON and iN, as opposed to only iN, are better correlated with vegetation stand height and forest N-requirements, thus DON is important for plant nutrition. We suggest that the gradients of iN and oN bioavailability, e.g., high iN near vertebrate colonies but high oN further inland, reflect the N-form predominantly acquired by sub-Antarctic plants.

Direct oN uptake by plants has energetic benefits due to the simultaneous acquisition of C with N and can affect plant growth (Franklin et al. 2017). However, contrary to other literature showing increased RGR and root allocation with oN (e.g., Cambui et al. 2011; Franklin et al. 2017), we found decreased RGR on oN and low root:shoot ratios with high (4 mM) oN supply. Biomass allocation responses to different N-forms may be concentration- and compound-specific.

For example, root inhibition and stimulation of *A. thaliana* has been documented, but depends on the amino acid concentration and type supplied, as well as the specific ecotype under investigation (Walch-Liu et al. 2006; Lonhienne et al. 2014). Here, we detected species-specific responses in root:shoot ratio and leaf N, where *P. cookii* leaf N increased with root fraction but *P. annua* leaf N decreased with root fraction. The other species showed significant responses to the treatments, where *A. stolonifera* increased leaf N with high N concentrations, and *P. magellanicus* showed higher root growth with NO_3^- relative to glycine. This suggests a greater complexity to growth responses under different N-forms than that presented by Franklin et al. (2017), varying between species, concentrations, and N compounds. Plant growth strategies and their growing environment likely play a more important role in growth and allocation responses.

The different species responses to N-form and N concentration may reflect different life history strategies and thus the different environments they inhabit. For example, root proliferation under low N (irrespective of the N-form), as shown with *P. annua*, suggests nutrient foraging in otherwise limiting conditions (Miller and Cramer 2004). *Poa annua* is a ruderal alien with a near global distribution (Govaerts et al. 2021). In the Antarctic, its invasion success has been linked to high plasticity in photosynthetic traits and biomass allocation, and strong competitive ability for nutrient acquisition compared to native species (Molina-Montenegro et al. 2016; Cavieres et al. 2018; Rudak et al. 2019; Ripley et al. 2020). Contrasting *P. annua* biomass allocation, *P. magellanicus* root allocation was strongly affected by N-form irrespective of total N concentration. The combination of treatments influenced allocation and leaf N for *P. cookii*, with high leaf N and root fractions at 4 mM NO_3^- but low leaf N and root fractions at 4 mM glycine. Root proliferation under high N concentrations is a competitive strategy for N capture (Robinson et al. 1999; Hodge 2009). In an environment rich in oN with sporadic faunal iN input, responses to N-forms may confer a competitive advantage, especially considering that iN led to higher RGR than oN. These different growth strategies may influence species interactions and co-existence in the field, such as resource competition. For example, high N acquisition and biomass allocation under low N by *P. annua* may result in competition with native flora. The use of different N-forms and how they affect plant growth in natural settings thus represents an important avenue for future research.

Most work on Marion Island terrestrial N (and nutrient) cycling dates to the late twentieth century, resulting in a paucity of contemporary data under current paradigms and global change. It is important to quantify the extent to which oN acquisition meets plant N budgets to fully understand soil–plant–N fluxes and terrestrial energy flow on Marion Island under anthropogenic driven change such as climate warming and terrestrial invasions. Rates of biological invasions in the sub-Antarctic are high (le Roux et al. 2013; Greve et al. 2017) and may have cascading influences on the system. For example, the predation of soil invertebrates by the invasive house mouse limits nutrient cycling (Smith 2002; McClelland et al. 2018). Moreover, *P. annua* shows a high capacity for N acquisition, which may contribute to further expansions of this species. Understanding N sources utilised by plants is also important given the evidence for current warming in the region (le Roux and McGeoch 2008a). Soil warming may alter the relative availability of oN and iN and thus the N-form predominantly acquired by plants; for example, Kuster et al. (2016) show warming increasing soil iN availability and thus plant iN uptake. This may have important implications for vegetation N and C

demands due to the differences in plant NUE between these N-forms (Franklin et al. 2017) and the different growth responses shown here. Alterations in N-form availability may also result in species composition changes, should certain species compete better for different N-forms (Pang et al. 2019). For example, warming in the maritime Antarctic and subsequent increases in moss decomposition allows the vascular plants to directly access oN in the form of proteins and peptides (Hill et al. 2019). Higher oN availability, and the mycorrhizal symbionts involved in oN uptake, is thought to facilitate the recent replacement of moss communities by vascular plants (Hill et al. 2019). Marion Island invasive flora have shown recent range expansions under climate warming (le Roux and McGeoch 2008b; le Roux et al. 2013). The role of N concentration and N-form availability in plant range expansions warrant further investigation, particularly following evidence for topographic and edaphic limitations to range expansions on Marion Island (Cramer et al. 2022).

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Author contributions NCMP, MDC, MG, and BSR conceived and designed research. NP conducted experiments, analysed data, and wrote the manuscript. All authors read and approved the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

Ethical approval This study is based on plant data and did not require ethics approval.

Informed consent This study is based on plant data and did not require informed consent.

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