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Abstract

Mosses play key ecological roles in water and nutrient retention in many ecosystems, yet relatively little is known of the functional characteristics of moss species, particularly nutritional characteristics. We investigated net flux of ammonium, nitrate and protons, using a microelectrode ion flux measurement system, in the gametophytes of 21 common moss species from three contrasting locations in south coastal British Columbia. The general location from which mosses were collected did not significantly affect ammonium or nitrate uptake. Proton efflux was greatest in mosses from locations with high rainfall. Rates of nitrate uptake differed among moss families, but there were no significant differences in uptake among species within families. Ammonium net flux differed among moss families, but also among species nested within family, with some species showing uptake and other showing ammonium efflux. In general, moss species native to dry habitats appeared to have higher rates of nitrogen uptake when ammonium and nitrate were available under favourable conditions.

Key words

bryophytes, nitrogen uptake, ion flux measurement

Introduction

Despite their small stature, mosses play key ecological roles in water and nutrient retention in many ecosystems (Turetsky 2003; Boy et al. 2016). The low thermal conductivity, high porosity and high water holding capacity of mosses effectively buffer moss-covered substrates from variation in atmospheric conditions (Turetsky et al. 2010).

Mosses also play an important role in carbon and nitrogen (N) cycling in many ecosystems by (i) addition of N to the ecosystem via N-fixing associations with cyanobacteria (DeLuca et al. 2002), (ii) sequestration of fixed and deposited N in moss tissues, and (iii) production of recalcitrant tissues that resist decomposition (Lang et al. 2009; Turetsky et al. 2010).

Mosses are one of the most ancient groups of land plants, with an estimated age of divergence from liverworts of 470-475 million years before present (Magallón et al. 2013; Liu et al. 2014), and the oldest fossils dating from the Mississippian of eastern Germany (Hübers and Kerp 2012), ~ 345 million years before present. With roughly 20,000 extant species, mosses are second only to angiosperms in diversity, and are a dominant feature of many moist temperate, montane, boreal and arctic ecosystems (Ayers et al. 2010). Mosses have followed a different evolutionary trajectory from vascular plants, and, for the most part, are small, poikilohydric and desiccation tolerant (Proctor 2000; Shaw and Goffinet 2000; Turetsky 2003). These attributes allow mosses to colonize a wide variety of substrates, ranging from humus to rock (Proctor 2000) although most have lost the ability to associate with mycorrhizae to assist in nutrient uptake (Wang and Qiu 2006). Mosses never developed roots or vascular tissue, therefore water and nutrient uptake occur over the entire plant surface, but most nutrient uptake occurs through the leafy tissues rather than through the rhizoids (Turetsky 2003).

As for most plants, N is the mineral nutrient required in the greatest quantity by many mosses. Mosses acquire N in three main ways: from deposition and throughfall, from the soil, and from associated cyanobacteria (Shaw and Goffinet 2000; Rousk et al. 2013). For most moss species, the majority of N is derived from deposition and throughfall on moss gametophyte tissues (Ayres et al. 2006). Only some moss species associate with cyanobacteria, and N obtained from fixation by these cyanobacteria is important in regions with low rates of atmospheric N deposition (Turetsky 2003). Recently, it has been shown that both endohydric and ectohydric mosses utilize soil N (Ayres et al. 2006). Nevertheless, soil N fulfills only a fraction of the moss N budget, and for most species, N deposition and throughfall are the main source of N (Ayres et al. 2006).

Mosses lack a fully developed cuticle and so are more able to take up nutrients through leaf and stem surfaces than are vascular plants. Mosses can accumulate N in situations of high N supply from deposition and throughfall (Limpens and Berendse 2003;

Wiedermann et al. 2009), and alterations in N availability can lead to changes in moss physiology, growth and competitive interactions (Soares and Pearson 1995; Gundale et al. 2013).

Mosses take up a diverse range of N forms, including both organic and inorganic N, and can sequester N, making it unavailable for vascular plant uptake in some conditions (Turetsky 2003; Liu et al. 2013; Rousk et al. 2013). Like many plants, mosses often take up ammonium preferentially over other N forms (Jauhiainen et al. 1998; Turetsky 2003; Liu et al. 2013; Wang et al. 2014); however, the ability to assimilate ammonium and to avoid cellular toxicity when N is supplied as ammonium is an important components of species' N preference (Britto and Kronzucker 2013). In addition to inorganic N uptake, some moss species also show a capacity for high rates of organic N uptake (Song et al. 2016).

Given the high species diversity of mosses and the wide diversity of ecological habitats they occupy (Rose et al. 2016), moss species might be expected to differ physiologically. Relatively little is known, however, about diversity in functional traits among mosses (Jonsson et al. 2015). N uptake, N form preferences and N assimilation play important functional roles in other plant species, and these characteristics could be expected to also differ among moss species, however, variation in nutritional characteristics among moss species remain largely unexplored. The objectives of this study were: (i) to measure inorganic N uptake rates and N form preferences in a sample of 21 common mosses from a range of locations in coastal British Columbia, the region with the greatest number of moss species in Canada, and (ii) to relate N uptake rates to the species' ecological characteristics.

Materials & Methods

Twenty-one common moss species from 12 families were sampled from three locations on southern Vancouver Island, British Columbia: Victoria (48.4 ^{o}N, 123.4 ^{o}W, 5-200 m), Port Renfrew (48.5 ^{o}N, 124.4 ^{o}W, 10-250 m) and Bamfield (48.8 ^{o}N, 125.1 ^{o}W, 20 m). Victoria is in the Coastal Douglas-fir biogeoclimatic zone and has a dry summer with less than 40 mm of rain per month from May to September (Government of Canada 2017). Victoria mosses were collected under second-growth Douglas-fir (*Pseudotsuga menziesii*

(Mirb.) Franco) or Garry oak (*Quercus garryana* Douglas ex Hook.) overstorey, from forest floor, tree trunk or rocky substrates. Port Renfrew and Bamfield sites are in the Coastal Western Hemlock biogeoclimatic zone, one of the highest rainfall regions of the province. Mosses in these locations were collected from second-growth forests under mixed overstories of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) western redcedar (*Thuja plicata* Donn ex D. Don) and Douglas-fir, from the forest floor or from decomposing logs. In each location, between three and fifteen samples containing approximately 10-50 gametophores of each moss species were collected and later identified with the assistance of experienced bryologists (Table 1). Once collected, samples were kept in clear plastic containers covered by a lid, and were maintained in a growth chamber at 20°C, with 16 hours of light per day. Mosses were misted with distilled water at least twice per week and were maintained in these conditions for at least three days before measurement.

Nitrogen form uptake was measured on the leafy tissue of all moss species using a microelectrode ion flux measurement system (MIFE, Unitas Consulting, Hobart, AU). At least three, separate gametophores of each moss species from each sample were chosen for flux measurements. We attempted to simulate the natural conditions in which mosses take up N through their gametophyte tissues. Therefore, we adjusted the pH of the measuring solution to the pH of open-sky rainwater in the Victoria area (~ pH 7.0) using 1.0 M NaOH and 1.0 M H_2SO_4. Each gametophore was measured within 1 cm of the top of the shoot and an intact, representative leaf was chosen for measurement.

Prior to measurement, intact moss gametophores were tied to a Perspex strip and immersed in a test tube in 60 mL of 500 μM NH_4NO_3 + 200 μM $CaSO_4 \cdot 2H_2O$ solution for 30-60 minutes. Aeration was maintained using a bubbler. The sample was removed from the test tube and transferred to a tray filled with the same solution.

Measurements of ion net flux were performed as per Hawkins and Robbins (2010). Net flux of protons, ammonium (NH_4^+), and nitrate (NO_3^-) were measured. Electrode blanks were pulled from 1.5 mm borosilicate glass capillaries, dried in an oven at 220°C for 5 h, and silanized with tributylchlorosilane (catalog no. 90796, Fluka, Sigma-Aldrich Canada Ltd., Oakville, ON). Cooled microelectrodes were backfilled with 200 mM NH_4Cl for NH_4^+,

500 mM KNO_3 plus 100 mM KCl for NO_3^-, and 15 mM NaCl plus 40 mM KH_2PO_4 (adjusted to pH 6.0 using 0.1 M NaOH) for H^+. Electrode tips were then filled with commercially available ion-selective H^+ or NH_4^+ cocktails (Fluka catalog no. 95297 and 09882, respectively), or a NO_3^--selective cocktail containing 0.5% methyltridodecylammoniumnitrate, 0.084% methyltriphenylphosphonium bromide and 99.4% n-phenyloctylether (Plassard et al. 2002). Electrodes were calibrated with a set of known standards. The electrodes were mounted on an electrode holder (MMT-5, Narishige, Tokyo, Japan) providing three-dimensional positioning. Electrodes were positioned in a line 5-30 µm above the leaf surface with their tips spaced 3–4 µm apart. The chamber was attached to a computer-controlled micromanipulator (PatchMan NP2, Eppendorf AG, Hamburg, Germany). During flux measurements, the MIFE computer gently moved the chamber up and down, providing virtual movement of the electrode tips between two positions 40 µm apart, in a 10 s square-wave cycle. The concentration of each ion was calculated from its electrochemical potential at each position. The flux of each ion was later calculated from the measurements of the difference in the electrochemical potential between these positions (Shabala et al. 1997).

Thirty-five of the 207 total moss samples collected from the three locations had a sufficient quantity of gametophyte remaining after gametophores were removed for ion flux analysis to analyze the samples for N and carbon (C) concentration. Bulked gametophytes of one to six samples per species (Table 4) were ground to a powder in a Wig-L-Bug amalgamator (Crescent Dental Mfg. Co., Lyons, Ill.), and analyzed for N and C content using a Flash EA 1112 elemental analyzer (ThermoQuest Italia S.p.A., Rodano, Italy).

Net fluxes of NH_4^+, NO_3^- and H^+ in the 21 moss species were analyzed with General Linear Model ANOVAs to determine differences in net flux, firstly, among species, locations and their interaction, and secondly, among families and species nested within families. All statistical tests were carried out with SAS PROC GLM using Type III Sums of Squares for unbalanced experimental designs. Before analysis, normality was assessed with Ryan-Joiner tests and homogeneity of variance was assessed with Levene's test. To determine differences among means, Least Significant Difference tests were used. Mean N and C

concentrations for the 20 moss species x location combinations were correlated with mean NH_4^+ and NO_3^- net fluxes for the respective species and locations using SAS PROC CORR.

Results

Moss species differed significantly in net fluxes of NH_4^+, NO_3^- and H^+ (Table 2). The location from which mosses were collected did not significantly affect N uptake, but did significantly affect net proton flux (Table 2). Proton efflux was greatest in mosses from Port Renfrew (-41.1 $\pm$ 5.4 nmol m^{-2} s^{-1}), intermediate in mosses from Bamfield (-33.4 $\pm$ 8.0 nmol m^{-2} s^{-1}) and least in Victoria mosses (-15.8 $\pm$ 3.1 nmol m^{-2} s^{-1}). The species x location interaction term was not significant for net proton flux, but the near-significance of the interaction for net NH_4^+ and NO_3^- flux (Table 2) suggests caution in the interpretation of results. For those species that were found in more than one location, the significant interaction resulted from variation in the magnitude of the differences in net flux between locations for each species, not from large changes in the ranking by location.

Comparing ion flux data among species grouped by family showed moss families to differ significantly in net fluxes of all ions (Table 3). The effect of species nested within family was significant for NH_4^+ flux, only (Table 3). Rates of NO_3^- uptake were consistent within families, with species in Grimmiaceae and Leucodontaceae having high rates of NO_3^- uptake, and *Sphagnum rubiginosum* having the lowest rate of NO_3^- uptake (Figure 1). NH_4^+ net flux was more variable among species, with some species taking up NH_4^+, and others showing NH_4^+ efflux (negative flux) (Figure 1). Grimmiaceae, Leucodontaceae and *Metaneckera menziesii* had high NH_4^+ uptake, while Hylocomiaceae demonstrated consistent NH_4^+ efflux (Figure 1). Many moss families and species showed high and variable H^+ efflux (Figure 1). In contrast, the two species in Leucodontaceae, the three species in Mniaceae, *Metaneckera menziesii* and *Orthotrichum lyellii* had very low H^+ efflux (Figure 1).

The two species of *Racomitrium,* plus *Dendroalsia abietina* and *Antitrichia californica* all had relatively high rates of uptake of both N forms (Figure 1). In contrast, the two species of *Polytrichum* and the three species of *Dicranum* had relatively high NO_3^- uptake but low

NH_4^+ uptake (Figure 1). *Metaneckera menziesii* appeared to have a significant preference for NH_4^+ over NO_3^- (Figure 1).

The 35 moss samples with sufficient material for N and C analyses exhibited a range of N concentrations (Table 4). N concentrations ranged from 2.93 % in *Kindbergia oregana* to 0.75 % in one sample of *Dendroalsia abietina*. C concentrations ranged from 48.9 % in *Dicranum scoparium* to 42 % in *Rhizomnium glabrescens* (Table 4). There were no significant correlations of N or C concentrations with mean NO_3^- or NH_4^+ net flux from the corresponding samples.

Discussion

Mosses of the same species collected from different locations had similar NO_3^- and NH_4^+ net fluxes, even though populations of some species originated from locations with very different climates. This suggests that N uptake characteristics of moss species relate to acclimation to common microhabitat characteristics, or functional differences among species related to their microhabitat. Location and species both had a significant effect on proton net flux, with mosses from high rainfall locations having greater proton efflux, on average, than mosses from the drier, Victoria area. The high proton efflux observed in many moss samples has also been observed in plant roots, associated with NH_4^+ uptake (Hawkins and Robbins 2010). We speculate that moss tissues that are commonly hydrated could have more, or more active, proton transporters to facilitate ion uptake from moist tissue surfaces. Similarly, most of the moss species with an epiphytic habit on tree trunks, which presumably would be relatively dry (*Dendroalsia abietina, Antitrichia californica, Metaneckera menziesii* and *Orthotrichum lyellii*) had relatively low proton efflux.

Patterns of NH_4^+ and NO_3^- uptake were less consistent than those observed for plant roots or mycorrhizae measured with the MIFE system. For moss species exhibiting NH_4^+ uptake, rates of NH_4^+ uptake were generally much greater than rates of NO_3^- uptake, as has been found in other moss species (Jauhiainen et al. 1998; Turetsky 2003; Liu et al. 2013; Wiedermann et al. 2009; Wang et al. 2014). Relatively high NH_4^+ uptake could be due to both the high cation exchange capacity of mosses and the inhibition of NO_3^- uptake in the

acidic environments favoured by mosses (Turetsky 2003; Liu et al. 2013). For some moss species, however, consistent NH_4^+ efflux was observed. Two of the moss species with significant NH_4^+ loss are in the family Hylocomiaceae, which includes species associating with N-fixing bacteria (Bay et al. 2013). If N-fixing species had excess N supply, some NH_4^+ might be lost to the incubation solution, however, significant NH_4^+ loss was also observed from *Polytrichum commune* and *Dicranum tauricum*, species which do not form associations with N-fixing bacteria (Basilier 1979; Bay et al. 2013). Also, the two species in the Hylocomiaceae did not exhibit notably high N concentrations in their tissues.

NO_3^- uptake was observed in all moss species, except *Sphagnum rubiginosum*. It is notable that the *Sphagnum* species, typically from N-poor environments, had very low rates of NO_3^- and NH_4^+ uptake, especially in light of the knowledge that some *Sphagnum* species have a high capacity for N fixation (Markham 2009). The low rates of N uptake per unit leaf area in *Sphagnum* may be attributed to the relatively low proportion of living cells in leaves of this genus. In contrast, species from the Grimmaceae and Leucodontaceae had the highest rates of NO_3^- uptake and the greatest total N uptake. The two species of *Racomitrium* (Grimmaceae) are the only species in this study typically found on open, dry, gravelly or rocky substrates and the two species in the Leucodontaceae are epiphytes on oaks or *Arbutus* in dry environments. We speculate that moss species native to very dry habitats may be able to take up N, particularly NO_3^-, at higher rates when conditions become moist and favourable for nutrient uptake.

While differences in N form uptake rates among moss species are significant, N uptake measured in solutions of uniform N concentration and pH tells only part of the story of moss nutrition. N form uptake rates are affected by N supply, pH and temperature (Britto and Kronzucker 2013). The N form absorbed also affects the physiology, and energetic and C costs of N assimilation, as well as extra- and intracellular pH (Britto and Kronzucker 2013). Despite the complexity created by interacting physiological and environmental variables, assessing N form uptake under controlled conditions is important to understanding moss nutrition. In many ways, mosses are an ideal group in which to study nutritional complexity, as their lack of a complete cuticle, high cell wall cation exchange

capacity and reliance on N uptake over the surface of the gametophore allows more direct measurement of the physiology of N uptake, compared to vascular plants with roots.

Surprising consistency in rates of NH_4^+ and NO_3^- uptake within the moss species or families found in two contrasting climates suggests either that mosses are acclimated to their microhabitat in terms of N uptake characteristics, or that differences are phylogenetic. Functional differences in nutritional characteristics among moss species would, in part, explain their individual habitat affinities and the wide range of habitats occupied by mosses. Relative to vascular plants, little is known about bryophyte functional characteristics and what factors control bryophyte communities (Jonsson et al. 2015). In the boreal forest of northern Sweden, measures of moss community composition and functional traits were primarily influenced by the vascular plant community composition and productivity, and abiotic factors had only an indirect effect (Jonsson et al. 2015). In our study, it appeared that phylogenetics, or abiotic factors of moss microhabitats, such as moisture availability, had a strong influence on N uptake. Understanding the capacity of moss species for N uptake and sequestration is important as these characteristics could strongly influence ecosystem productivity in low N environments such as cool temperate and boreal forests where mosses predominate.

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Table 1. Number of samples analyzed for each moss species in each location (V = Victoria, PtR = Port Renfrew, B = Bamfield) and common habitat of each species (Flora of North America, 1993+)

Class, Family, Species	V	PtR	B	Habitat
Bryidae				
Brachytheciaceae				
Kindbergia oregana (Sull.) Ochyra	3	-	3	litter, soil rich in humus, rotten logs
Isothecium stoloniferum (Mitt.) Ren. and Card.	5	15	-	epiphytic on tree trunks, branches, shrubs, exposed or shaded rock cliffs, boulder slopes
Dicranaceae				
Dicranum fuscescens Sm.	8	-	6	common on wood and humus, occasionally epiphytic on living trees
Dicranum scoparium Hedw. Sp. Musc. Frond.	-	4	-	soil, humus, humus over rock, decaying stumps and logs, tree bases in dry to mesic woodlands
Dicranum tauricum Sap.	12	-	-	frequently on rotten logs, stumps, or tree bases in woodlands, sometimes on humus
Grimmiaceae				
Racomitrium elongatum Ehrh. ex Frisvoll	3	-	-	dry, exposed areas, sandy soil
Racomitrium lanuginosum (Hedw.) Brid.	3	-	-	dry, exposed areas, acidic soil and rocks
Hylocomiaceae				
Rhytidiadelphus loreus (Hedw.) Warnst.	-	5	-	especially common on logs, but also soil, humus, rock, and base of trees
Rhytidiadelphus triquetris (Hedw.) Warnst.	15	-	-	soil, humus, less often on logs and rock
Hypnaceae				
Buckiella undulata (Hedw.) Ireland	-	15	-	logs, stumps, base of trees, boggy soil, soil and humus overlying rock
Leskeaceae				
Claopodium crispifolium (Hook.) Ren. & Card.	-	4	-	rotten logs, tree bases, soil over rock
Leucodontaceae				
Antitrichia californica Sull.	11	-	-	bark, decorticated wood, on *Quercus*, siliceous rock, humic soil, full sun or partial shade
Dendroalsia abietina (Hook.) Britt.	5	-	-	tree trunks, limbs, and branches, large rock surfaces
Mniaceae				

Leucolepis acanthoneura (Schwaegr.) Lindb.	-	5	5	soil, boulders, rotten logs, tree trunks, shaded habitats
Plagiomnium insigne (Mitt.) Kop.	13	-	3	humus or soil in shaded habitats in forests
Rhizomnium glabrescens (Kindb.) Kop.	15	5	4	logs or tree bases, moist soil near streams, thin soil over shaded rock
Neckeraceae				
Metaneckera menziesii (Hook. ex. Drumm.) Steere	8	-	7	epiphytic, especially on trunks of *Acer*, frequent on shaded, dry rock surfaces
Orthotrichaceae				
Orthotrichum lyellii Hook. & Tayl.	6	-	-	trees, covering trunks to 10 m, boulders
Polytrichaceae				
Polytrichum commune Hedw.	4	-	6	moist, organic soil in wet habitats, peatlands, margins of bogs or swamps
Polytrichum juniperinum Hedw.	5	-	-	exposed, well-drained, mostly acid soils, on thin shallow soil overlying rocks
Sphagnidae Sphagnaceae				
Sphagnum rubiginosum Flatberg, Lindb.	-	-	4	shaded areas in humid spruce forests

Table 2: General linear model ANOVA results (Type III sums of squares) of net fluxes of NH_4^+, NO_3^- and H^+ from 21 moss species from three locations

Source of Variation	df	NH_4^+ MS	NH_4^+ *Pr > F*	NO_3^- MS	NO_3^- *Pr > F*	H^+ MS	H^+ *Pr > F*
Species	20	4651	<0.0001	271	<0.0001	3454	<0.0001
Location	2	1189	0.21	218	0.11	8296	0.002
Species x loc.	6	1432	0.09	179	0.08	1299	0.42
Error	175	762		94		1283	

Table 3: General linear model ANOVA results (Type III sums of squares) of NH_4^+, NO_3^- and H^+ net flux by family and species nested within family for 21 moss species from 12 families

Source of Variation	df	NH_4^+ MS	NH_4^+ *Pr > F*	NO_3^- MS	NO_3^- *Pr > F*	H^+ MS	H^+ *Pr > F*
Family	11	6486	<0.0001	490	<0.0001	5131	<0.0001
Species(fam)	9	2078	0.007	128	0.22	2110	0.13
Error	195	788		97		1365	

Table 4: Mean (± S.E.) nitrogen (N) and carbon (C) concentrations in bulked gametophytes of 15 moss species from three locations (*n* = number of samples)

Family and Species	Victoria			Pt. Renfrew			Bamfield		
	N (%)	C (%)	*n*	N (%)	C (%)	*n*	N (%)	C (%)	*n*
Brachytheciaceae									
Kindbergia oregana	2.93	43.55	1						
Isothecium stoloniferum	1.25	44.15	1	0.98 (0.001)	44.01 (0.34)	6			
Dicranaceae									
Dicranum fuscescens	1.80	44.83	1				1.09 (0.03)	43.65 (0.30)	2
Dicranum scoparium				1.47	48.93	1			
Hylocomiaceae									
Rhytidiadelphus triquetris	1.45 (0.29)	44.31 (0.23)	3						
Hylocomiaceae									
Rhytidiadelphus loreus				1.22	44.94	1			
Hypnaceae									
Buckiella undulata				1.14 (0.05)	45.60 (0.86)	3			
Leskeaceae									
Claopodium crispifolium				1.33	44.25	1			
Leucodontaceae									
Antitrichia californica	1.03 (0.001)	44.21 (0.18)	2						
Dendroalsia abietina	0.93 (0.18)	44.53 (0.61)	2						
Mniaceae									
Leucolepis acanthoneura				1.67	45.02	1			
Plagiomnium insigne	2.36 (0.23)	42.89 (0.69)	2				1.07	43.62	1
Rhizomnium glabrescens	1.66 (0.12)	43.07 (0.34)	2	1.24	42.2	1	1.07	44.20	1
Neckeraceae									
Metaneckera menziesii	1.35	45.38	1						
Polytrichaceae									
Polytrichum commune							1.25 (0.11)	43.94 (0.68)	2

Figure 1. Mean net flux (± S.E.) of NO_3^-, NH_4^+ and H^+ in 21 moss species grouped into their respective families. Species codes are the first two letters of the genus and species names. Upper case letters indicate significant differences in ion flux among families ($p \leq 0.05$).

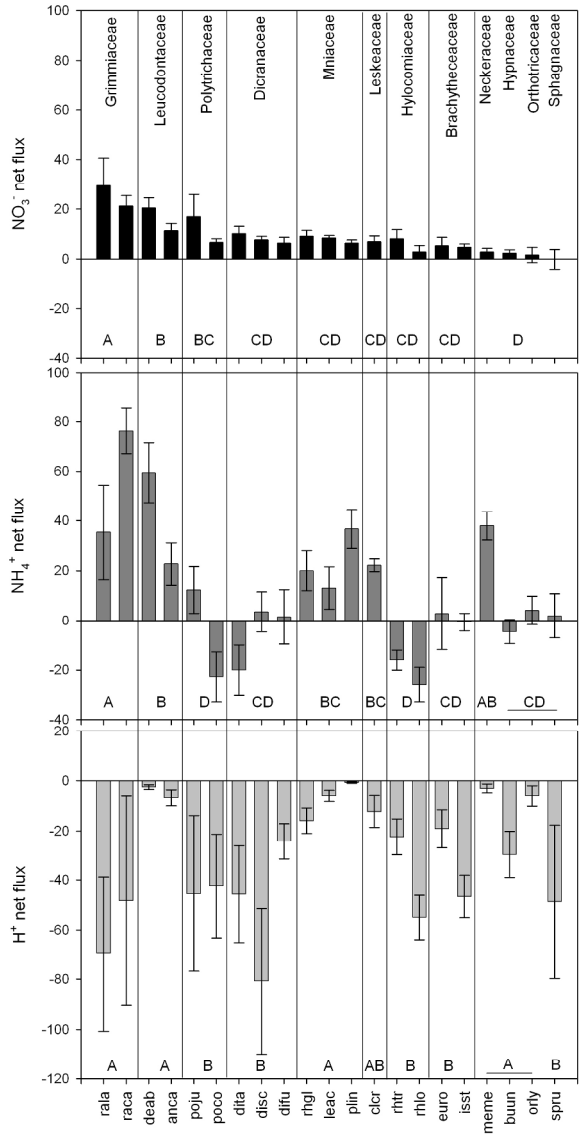

Figure 1. Mean net flux (± S.E.) of NO_3^-, NH_4^+ and H^+ in 21 moss species grouped into their respective families. Species codes are the first two letters of the genus and species names. Upper case letters indicate significant differences in ion flux among families ($p < 0.05$).

431x558mm (150 x 150 DPI)