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Fungal colonization patterns and enzymatic activities of peatland ericaceous plants following long-term nutrient addition

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1 **Fungal Colonization Patterns and Enzymatic Activities of Peatland**

2 **Ericaceous Plants Following Long-Term Nutrient Addition**

3

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24

25 **Abstract**

26 Northern peatlands are often dominated by ericaceous shrub species
27 which rely on ericoid mycorrhizal fungi (ERM) for access to organic
28 sources of nutrients, such as nitrogen (N) and phosphorus (P), and host
29 abundant dark septate endophytes (DSE). Relationships between hosts
30 and fungal symbionts may change during deposition of anthropogenic N
31 and P. We studied the long-term effects of N and P addition on two
32 ericaceous shrubs, *Calluna vulgaris* and *Erica tetralix*, at Whim Bog,
33 Scotland by analyzing fungal colonization of roots, enzymatic activity,
34 and fungal species composition. Unexpectedly, the frequency of typical
35 ERM intracellular colonization did not change while the occurrence of
36 ERM hyphae tended to increase and DSE hyphae to decrease. Our
37 findings indicate that altered nutrient limitations shift root associated
38 fungal colonization patterns as well as affecting ericaceous root enzyme
39 activity and thereby decomposition potential. Reduction of recalcitrant
40 fungal biomass in melanized DSE may have implications for peatland C
41 sequestration under nutrient addition.

42

43

44 **Keywords:** ericoid mycorrhizae; peatland; nutrient limitation; enzyme
45 activity; mycorrhizal colonization; dark septate endophyte; nitrogen
46 deposition; *Sphagnum*

47

48 Declarations of interest: none

49 1 Introduction

50 Peatlands in the Northern hemisphere are often nutrient poor
51 ecosystems characterized by acidic, anoxic, water saturated conditions
52 with considerable nitrogen (N) and phosphorus (P) limitations (Aerts et
53 al., 2001). These conditions and the accumulation of recalcitrant
54 vegetation litter containing high concentrations of phenolic compounds
55 and humic acids inhibitory to microorganisms and vegetation are
56 considered to largely suppress decomposition (Leake and Read, 1990;
57 Painter, 1991; Read et al., 2004; van Breemen, 1995). The challenging
58 conditions in peatlands support a unique diversity of vegetation, with
59 ericaceous species comprising one of the most dominant ground cover
60 groups. Ericaceous shrubs are largely dependent on ericoid mycorrhizal
61 fungi (ERM) to provide access to organic N and P which they provide in
62 exchange for photosynthetic carbon (C) from the host plant (Smith and
63 Read, 2008). The ERM fungi are capable of accessing organic N and P via
64 a large variety of degradative enzymes which act primarily on plant cell
65 wall components (Perotto et al., 2018), demonstrating a potential
66 versatility more comparable to saprotrophs than to other types of
67 mycorrhizae. These ericaceous species are also host to abundant dark
68 septate endophytes (DSE) with extracellular enzyme capabilities
69 potentially capable of improving host nutrient uptake (Mandyam and
70 Jumpponen, 2005, 2014; Upson et al., 2009).

71

72 Over the past 150 years atmospheric deposition of N and P in forms
73 easily accessible to plants has been increasing through combustion of
74 fossil fuels and agricultural fertilization (Galloway et al., 2013; Galloway
75 et al., 2003; Tipping et al., 2014; Wang et al., 2015). As nutrient
76 limitations are alleviated, ericaceous reliance on ERM fungi may be
77 reduced, potentially altering the symbiont community and leading to
78 the loss of mycorrhizal symbionts. As mycorrhizal symbionts likely play a
79 role in protection against pathogens (Vohník et al., 2016; Weiß et al.,
80 2016), host species may in turn become more vulnerable to infection.
81 Furthermore, the long-term effects of an altered nutrient balance in
82 peatlands may include reduced nutrient acquisition competitiveness for
83 ericaceous species. The ERM fungi are not as efficient decomposers as
84 free-living saprotrophs, which may be naturally kept in check by
85 nutrient limitations and direct competition with ERM fungi (Averill et al.,
86 2014). Reduction of nutrient limitations may free the saprotrophic
87 species' decomposition potential, leading to their dominance and the
88 decline of ERM species and their host plants.

89

90 The selective pressure of N deposition on the symbiont community
91 highlights a risk to peatland plant diversity, potentially leading to similar
92 large-scale community shifts as seen in the continental level decline of
93 ectomycorrhizal tree species and the increase in arbuscular tree species
94 described by Averill et al. (2018). Any large-scale changes to peatland
95 microbial and plant communities risk changing the status of peatlands

96 as net C sinks to net sources of greenhouse gas emissions (Andersen et
97 al., 2013), which holds a globally significant potential when considering
98 that peatlands sequester nearly one third of global soil organic carbon C
99 (Gorham, 1991). The risk to C sink potential has been indicated by
100 Larmola et al. (2013), who found that ecosystem C uptake did not
101 increase in a long-term nutrient addition experiment at a nutrient-poor
102 peatland in Canada simulating atmospheric N deposition.

103

104 The Whim Bog experimental site, located in the Scottish Borders, was
105 established in 2001 to study the effects of different N forms and P
106 addition on an ombrotrophic peat bog (Sheppard et al., 2004). This site
107 allowed us to study nutrient addition effects on the ericaceous species
108 *Calluna vulgaris* and *Erica tetralix* and their root associated fungi. The
109 primary goals of this study were to characterize the frequency and
110 morphology of mycorrhizal colonization in these ericaceous shrub
111 species, assess ericoid mycorrhizal root enzyme capability related to
112 organic matter degradation, and identify their fungal symbionts under
113 changing nutrient availability. We hypothesized that: (1) the frequency
114 of microscopically observed fungal colonization in ericaceous shrub
115 roots is reduced across both forms of N and NP nutrient addition
116 treatments, reflecting a reduction in reliance on symbionts for nutrient
117 uptake; (2) nutrient addition treatments alter root associated fungal
118 diversity, as determined by morphotypic analysis and ITS sequencing;

119 and (3) the activities of ericoid mycorrhizal root surface enzymes reflect
120 treatment nutrient limitations.

121

122 **2 Materials and Methods**

123 2.1 Study Site

124 The study site, Whim Bog, located in the Scottish Borders, UK (Latitude:
125 55.76670, Longitude: -3.26667), has undergone nutrient addition
126 treatment since 2001. The study site, Whim Bog, located in the Scottish
127 Borders, UK (Latitude: 55.76670, Longitude: -3.26667), has undergone
128 nutrient addition treatment since 2001. The four nutrient addition
129 treatments included in this study received annually 6.4 g N m^{-2} either as
130 sodium nitrate (NaNO_3) or as ammonium chloride (NH_4Cl), both with
131 and without P and K (as K_2HPO_4). K_2HPO_4 was added at a 1:14 P:N ratio
132 to represent the ratio found in amino acids (See Sheppard et al. (2004)
133 and Levy et al. (2019) for details). The ambient deposition in controls
134 was 0.8 g N m^{-2} . Precipitation collected at the site was mixed with
135 standard solutions to the required treatment concentrations. When
136 adequate precipitation was collected an automated sprayer-system
137 applied the treatment to the plots, simulating natural rainfall. Natural
138 precipitation was not excluded from plots. Plots received 15 years of
139 nutrient addition resulting in a 96 g N m^{-2} cumulative load.

140

141 2.2 Site Measurements and Sampling

142 In August 2016, plant species composition and abundance for each plot
143 were measured via the point-intercept method using a 0.36 m² frame on
144 permanent vegetation quadrats established on site. The frame was
145 placed at a height of ca. 1 m relative to the surface of the plot and a
146 graduated pin was used to measure the frame height and vertical
147 location of each vegetation point for 61 intercepts as described in
148 Larmola et al. (2013). Water table (WT) depth for each plot was
149 measured from holes present after extraction of ingrowth cores used in
150 a separate study, relative to moss surface height.

151

152 Triplicate plots per treatment and controls were sampled in November
153 2016 for *Calluna vulgaris* (L.) Hull and *Erica tetralix* (L.) plant roots from
154 one individual plant per plot. Fine root sections were collected from
155 several points throughout the root system of each sampled plant and
156 stored at 8 °C prior to transport, followed by storage at -20 °C. Each root
157 sample was split into two subsamples: one for microscopy and the other
158 for enzymatic and subsequent molecular analyses.

159

160 Surface peat from triplicate plots per treatment and controls was
161 sampled to a depth of 20 cm in each plot and stored at 8 °C prior to
162 transport, followed by storage at -20 °C. After melting, the pH of each
163 sample was measured following homogenization with deionized water
164 at a 1:4 ratio.

165

166 2.3 Microscopy

167 The mycorrhizal status of the ericaceous shrub species *C. vulgaris* and *E.*
 168 *tetralix* was determined via light microscopy and Trypan Blue staining as
 169 described in Kiheri et al. (2017). Using the magnified intersections
 170 method described by McGonigle et al. (1990), with one slide per species
 171 from each of three replicate plots per treatment and controls (15
 172 samples per species), using 300 counts per slide, mycorrhizal
 173 colonization was quantified according to different morphological
 174 categories, described in Table 1. These categories were used to estimate
 175 differences between the frequencies of potential ericoid mycorrhizal
 176 (ERM) hyphae and typical dark septate endophyte (DSE) hyphae. In our
 177 study, only those fungi which were robust in structure, heavily
 178 melanized and displaying septa were assessed to be DSE. This was
 179 necessary to prevent misclassification of those ERM species which
 180 produce melanized and septate hyphae (Vohník and Albrechtová, 2011).

181

182 Table 1. Microscopic morphological categories of ericaceous root

183 associated fungi.

Hyphal Type	Quantification Category	Morphological Characteristics
	ERM0/DSE0	No fungal presence
Potential ericoid mycorrhizae (ERM)	ERM1	Intracellular coiling
	ERM2	Intracellular hyphae
	ERM3	Colonizing surface hyphae
	ERM4	Extracellular surface hyphae
Dark Septate Endophyte (DSE)	DSE1	Intracellular coiling
	DSE2	Intracellular hyphae
	DSE3	Colonizing surface hyphae
	DSE4	Extracellular surface hyphae

184

185 Hereon, putative ericoid mycorrhizal morphotypes are referred to with
186 categories ERM1-4 and dark septate endophytes with DSE1-4 (Table 1).

187 Of the morphotypic categories, only ERM category 1 (intracellular
188 coiling) was interpreted as mycorrhizal colonization frequency.

189 Categories 2-4 were interpreted as potential changes in fungal diversity
190 and function.

191

192 2.4 Enzyme Assays

193 The enzymatic activities of root samples from *C. vulgaris* and *E. tetralix*
194 were determined using a multi-enzyme assay described by Pritsch et al.
195 (2011), originally performed for assessing ectomycorrhizal exo-enzyme
196 potential. From triplicate plots of each of the five treatments, rhizomes
197 of both ericaceous species were each sampled for 9 individual ca. 1 cm
198 root pieces, for a total of 270 root samples (n = 5 treatments x 3 plots x
199 2 species x 9 root samples). To measure eight different hydrolytic and
200 oxidative root surface enzyme potentials we used the method described
201 by Velmala et al. (2014) for fluorescences representing the potential
202 activities of leucine aminopeptidase (EC 3.4.11.1), hemicellulases via β -
203 glucuronidase (EC 3.2.1.31) and β -xylosidase (EC 3.2.1.37), cellulases via
204 cellobiohydrolase (EC 3.2.1.91) and β -glucosidase (EC 3.2.1.21),
205 chitinase via N-acetylglucosaminidase (EC 3.2.1.14), and acid
206 phosphatase (EC 3.1.3.2). Samples were incubated at room temperature
207 in the dark and under agitation at 180 rpm on a tabletop shaker, with

208 each root piece in individual wells of 96-well filter plates (30–40 μm
209 mesh size, AcroPrep™ 96 Filter Plate; PALL, Port Washington, NY, USA)
210 in buffers containing enzyme specific 7-amino-4-methylcoumarine
211 (AMC) or 4-methylumbelliferone (MU) substrates. Incubation times for
212 each enzyme followed the protocol of Pritsch et al. (2011). The
213 respective substrates used were Leucine-AMC, MU-xylopyranoside, MU-
214 β -D-glucuronide, MU-cellobiohydrofuran, MU-N-acetyl- β -D-
215 glucosaminide, MU- β -D-glucopyranoside, and MU-phosphate. After
216 incubation, substrate solutions were collected by centrifugation with a
217 96-well plate adapter at 3200 rpm onto Optiplate-96F reading plates
218 (Perkin-Elmer, Waltham, MA, USA) containing stop buffer (pH 10). Each
219 substrate's fluorescence was measured using a Victor³ 1420 multilabel
220 plate counter (Perkin-Elmer, Waltham, MA, USA) at an excitation
221 wavelength of 355 nm and an emission wavelength of 460 nm. Standard
222 solutions were prepared using aminomethylcoumarin (AMC) and 4-
223 methylumbelliferone (MUF) and used to calculate enzymatic activities
224 from concentrations of released AMC or MUF according to their
225 respective substrates. All standard and enzyme substrate solutions were
226 purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

227

228 Further, laccase (EC 1.10.3.2) activity was used as an indicator of lignin
229 modification activity and was determined by incubation in buffer
230 containing diammonium 2,2'-azinobis-3-ethylbenzothiazoline-6-
231 sulfonate (ABTS) and colorimetric measurement using a Tecan Infinite

232 M200 PRO Multimode Reader (Tecan Trading AG, Männedorf,
233 Switzerland).
234
235 Following enzymatic measurements, the individual root pieces were
236 scanned at high resolution (650 dpi) and their surface area measured
237 using winRHIZO Pro (ver. 2017, Regent Instruments Inc.) software.
238 These surface area values were used to convert enzymatic activity to
239 $\text{pmol mm}^{-2} \text{min}^{-1}$.

240

241 2.5 Molecular Methods and Sequencing

242 From triplicate plots of each of the five treatments, rhizomes of both
243 ericaceous species were each sampled for 9 individual ca. 1 cm root
244 pieces, for a total of 270 root samples ($n = 5 \text{ treatments} \times 3 \text{ plots} \times 2$
245 $\text{species} \times 9 \text{ root samples}$). Following enzyme assays and winRHIZO
246 analysis on these samples, a randomized subset of 1 of 3 treatment
247 replicates was taken from the 270 samples for sequencing. These 90
248 samples, plus four repeated samples, were then directly amplified using
249 a Phire Plant Direct PCR kit's plant leaf protocol (Thermo Fisher
250 Scientific, Waltham, MA, USA) and PCR using the ITS1F (Gardes and
251 Bruns, 1993) and ITS4 (White et al., 1990) primer pair. Individual root
252 pieces were manually crushed using sterile pestles in provided dilution
253 buffer, in order to release fungal cells both on the root surface and
254 within the root structure, and 1 μl of each mixture was then used as a
255 template in a 20 μl PCR reaction. Cycling conditions for the Direct PCR

256 were: initial denaturation 98 °C 5 min, 40 cycles (98 °C for 5 sec, 57 °C
257 for 5 sec, 72 °C for 20 sec), and final extension 72 °C for 1 min. The
258 Direct PCR products were separated in a 2% agarose gel in 1X TAE buffer
259 at 120V for 2 hours and each ITS band excised and purified using a
260 Nucleospin® Gel and PCR Clean-Up kit (Macherey-Nagel GmbH & Co.
261 KG, Düren, Germany). The purified products were then further amplified
262 using a DreamTaq PCR mastermix (Thermo Fisher Scientific, Waltham,
263 MA, USA) by using 1 ul of Direct PCR product as the template in a 20 µl
264 DreamTaq PCR reaction with the same ITS1F-ITS4 primer pair. The
265 DreamTaq PCR was performed with the following program: initial
266 denaturation 95 °C for 3 min, 35 cycles (95 °C for 30 sec, 57 °C for 30
267 sec, 72 °C for 1 min), and final extension 72 °C for 10 min. This was done
268 as a quick method to identify common fungal species in each root
269 sample which could then be linked to their associated root surface
270 enzyme activities. These ITS products were Sanger sequenced using the
271 ITS4 primer (Macrogen Europe, Amsterdam, NL). This produced ITS
272 fragments ranging from 98 to 884 bp in length. This method resulted in
273 the successful sequencing of 60% of (57 out of 94) ITS products
274 (Supplementary File 1), of which 38 sequences were more than 200 bp
275 in length and of high enough quality required for NCBI Gen Bank
276 submission. The sequences are disposed to Gen Bank under the
277 accession numbers **MN059889-MN059927**. See Supplementary File 1
278 for all 57 FASTA sequences. The high proportion of sequencing failure
279 was likely due to mixed ITS products from multiple fungal species and

280 inhibitory chemistry. Sequence identification was performed using the
281 Unite massBLASter analysis (Nilsson et al., 2019) on manually trimmed
282 sequences. Analyzed sequences had 86-100% sequence similarity to
283 existing reference or representative sequences within the INSD or
284 Environmental databases and the most likely species hypotheses (SH)
285 were selected as their fungal identities. Functional roles were then
286 assigned to these fungal identities according to the web based FUNGuild
287 bioinformatic tool (Nguyen et al., 2016).

288

289 2.6 Statistical Analyses

290 Hereon, α level for statistical significance is defined as $p \leq 0.05$ and
291 indicative as $0.05 < p \leq 0.1$ in all cases, and the term significant
292 specifically indicates statistical significance as $p \leq 0.05$. All analyses were
293 calculated using values from treatment means ($n=3$). Differences
294 between treatments, for each host plant separately, were determined
295 by analysis of variance (ANOVA) on logarithmically transformed data
296 followed by pairwise comparisons using the parametric Tukey's HSD and
297 nonparametric Games-Howell post hoc tests, using IBM SPSS Statistics
298 25. Fungal-Enzyme activity profiles in Figure 8 were prepared using
299 OriginPro 2018 by assigning fungal identifications to enzyme activities
300 on an individual root piece basis.

301

302 The combined data for both host plants' mycorrhizal morphotype
303 categories ERM1-4 and DSE1-4 (Table 1), root enzyme activities, plot

304 level vegetation abundance, surface peat pH, and water table depth
305 were analyzed using correlation analyses. Correlation analyses were
306 performed using `rcorr`-function with Spearman rank based correlation
307 from package `Hmisc v4.1-1` (Harrell et al., 2014) and plotted using
308 `corrplot`-function from package `corrplot v0.84` (Wei and Simko, 2016) in
309 the R programming environment (R Core Team, 2017).

310

311 **3 Results**

312 3.1 Vegetation, Peat pH, and Water Table

313 In all four nutrient addition treatments, the dominant ericaceous shrub,
314 *Calluna vulgaris*, tended to decrease in abundance while *Erica tetralix*
315 tended to increase, especially in NaNO_3 treatments (Table 2). Nutrient
316 addition treatments showed decreasing trends in the abundance of
317 *Sphagnum*, when compared with controls. The sedge *Eriophorum*
318 *vaginatum* showed increasing trends in abundance in all treatments
319 except NaNO_3 , when compared with controls. The high abundance of
320 reported *E. vaginatum* in the NaNO_3 +PK treatment was largely an effect
321 of one plot where the point-intercept measurements were performed
322 within a large *E. vaginatum* tussock. Furthermore, the different forms of
323 N addition were found to cause opposite changes in peat pH, with
324 NaNO_3 increasing pH by ca. 0.2-0.3 units and NH_4Cl decreasing pH by ca.
325 0.2-0.4 units (Table 2). Effects of treatments on peat pH were found to
326 be statistically significant ($F_{(4,10)}=6.410$), with pairwise comparisons
327 finding statistically significant differences between NaNO_3 and NH_4Cl +PK

328 treatments and between NaNO₃+PK and NH₄Cl+PK treatments. Mean
 329 water table (WT) depth, measured relative to moss surface, was eight
 330 cm closer to the moss surface in NaNO₃+PK treatments than in control
 331 plots, while NH₄Cl treatments affected WT the least. This was likely
 332 affected by the loss of moss abundance and subsequent subsidence in
 333 NaNO₃+PK treatments.

334

335 Table 2. Vegetation abundance (hits per m²), surface (0-20cm) peat pH,
 336 and treatment water table (WT) depth with ±1 standard deviation, n=3.
 337 Different superscript letters indicate significant differences (P<0.05)
 338 compared with the other treatments. Statistically significant differences
 339 were only observed for pH values ($F_{(4,10)}=6.410$).

	<i>Calluna vulgaris</i>	<i>Erica tetralix</i>	<i>Eriophorum vaginatum</i>	Other Vasc. spp.	<i>Sphagnum</i>	Other Mosses	pH	WT (cm)
Control	212.6 ± 33.6	4.0 ± 4.9	42.3 ± 32.0	2.0 ± 2.1	24.3 ± 17.5	28.3 ± 16.7	4.1 ± 0.0 ^{ab}	16.0 ± 12.4
NaNO₃	156.6 ± 72.3	57.0 ± 28.7	23.3 ± 2.3	5.6 ± 7.3	11.6 ± 16.4	25.3 ± 1.6	4.4 ± 0.2^a	12.6 ± 9.8
NaNO₃+PK	61.0 ± 41.4	11.0 ± 14.8	205.3 ± 182.1	5.0 ± 2.9	0.0	35.0 ± 11.3	4.3 ± 0.1^a	8.0 ± 1.4
NH₄Cl	221.6 ± 49.7	19.0 ± 12.3	57.0 ± 26.1	0.0	10.6 ± 5.2	20.3 ± 19.7	3.9 ± 0.1 ^{ab}	16.0 ± 2.6
NH₄Cl+PK	163.3 ± 72.6	24.6 ± 23.7	113.3 ± 64.5	1.3 ± 1.8	9.6 ± 8.01	24.6 ± 8.1	3.7 ± 0.1^b	24.0 ± 4.2

340

341 3.2 Root Associated Fungal Morphology and Frequency

342 The mean proportion of root intersects which were microscopically
 343 quantified as presenting fungal colonization increased from 78% and
 344 83% in control plots for *C. vulgaris* and *E. tetralix* roots, respectively, to
 345 89% and 91% in nutrient addition plots, respectively. Of these root
 346 intersections which contained fungal structures, both ericaceous host

347 species showed trends of increasing ERM hyphal frequency and
348 decreasing DSE hyphal frequency when under nutrient addition (Figure
349 1). Nutrient addition increased mean ERM hyphal frequency in *C.*
350 *vulgaris* and *E. tetralix* roots by 31% and 16%, respectively. However,
351 mean DSE hyphal frequency decreased by 30 % in *C. vulgaris* roots and
352 22% in *E. tetralix* roots. Though these trends are considerable, they
353 were not found to be statistically significant due to high natural
354 variation between samples.

355

356 Intracellular hyphal frequency (ERM2, Table 1) of *C. vulgaris* roots
357 significantly ($F_{(4,10)}=11.406$) increased by ca. two-fold under nutrient
358 addition when comparing controls to NaNO_3 +PK, NH_4Cl , and NH_4Cl +PK,
359 as well as increasing by approximately half under NaNO_3 addition, which
360 was a statistically indicative change (Figure 2). *C. vulgaris* roots showed
361 no statistically significant increases in cells containing typical ERM
362 intracellular coiling (ERM1), root surface colonizing hyphae (ERM3), or
363 extracellular surface hyphae (ERM4) under nutrient addition. *C. vulgaris*
364 DSE extracellular hyphal frequency (DSE4) decreased significantly under
365 NH_4Cl addition to less than 10% that of controls ($F_{(4,10)}=4.654$) (Figure 3).
366 The same analysis for *E. tetralix* roots showed similar trends of
367 increasing ERM morphotype frequencies under nutrient addition (Figure
368 2), while DSE morphotype frequencies decreased (Figure 3).
369 Interestingly, a statistically indicative positive correlation was found

370 between *E. tetralix* root surface DSE colonization frequency (DSE3) and
371 plot *Sphagnum* abundance ($r= 0.58$) (Figure 5).

372

373 3.3 Root Enzymatic Activity

374 The different forms of N addition, NH_4^+ and NO_3^- , induced variable and
375 opposing effects on C and N acquiring enzymes on the two ericaceous
376 shrubs' root surfaces, when compared to controls (Figures 6&7).

377 Treatment with NaNO_3 tended to reduce all *C. vulgaris* root enzymatic
378 activities except acid phosphatase, suggesting that *C. vulgaris* or its root
379 associated fungi are more sensitive to NaNO_3 than *E. tetralix*, which did
380 not show this effect. In *C. vulgaris* roots under NH_4Cl addition, the
381 activities of the C acquiring enzymes β -xylosidase and β -glucosidase did
382 not change compared to controls, while activity of β -glucuronidase
383 tended to increase two-fold and N-acetylglucosaminidase tended to
384 decrease by nearly half. In contrast, the effects of both forms of N on *E.*
385 *tetralix* roots were similar for all C and N acquiring enzymes, while
386 leucine aminopeptidase activity tended to be suppressed under NH_4Cl
387 addition to less than 25% of controls and N-acetylglucosaminidase
388 decreased by less than half, compared to controls. Laccase activity was
389 not detected in any samples.

390

391 Compared to control plots, addition of both forms of N alone tended to
392 induce an approximately 25% increase in acid phosphatase activity in
393 both plant species (Figures 6&7). In contrast, treatments with additional

394 PK reduced acid phosphatase activity to $1/3^{\text{rd}}$ of controls in both
395 ericaceous species (Figure 7). In *C. vulgaris* roots, the effect of nutrient
396 additions on acid phosphatase activity was statistically significant
397 ($F_{(4,10)}=8.163$), with significant differences between NaNO_3 and
398 NaNO_3+PK and $\text{NH}_4\text{Cl}+\text{PK}$ treatments, as well as between NH_4Cl and
399 NaNO_3+PK and $\text{NH}_4\text{Cl}+\text{PK}$ treatments. In *E. tetralix* roots the effect of
400 nutrient addition on acid phosphatase activity was significant
401 ($F_{(4,10)}=11.400$), with significant differences between control and
402 $\text{NH}_4\text{Cl}+\text{PK}$ treatments, between NaNO_3 and NaNO_3+PK and $\text{NH}_4\text{Cl}+\text{PK}$
403 treatments, and also between NH_4Cl and NaNO_3+PK and $\text{NH}_4\text{Cl}+\text{PK}$
404 treatments.

405

406 Interestingly, in both ericaceous species the NPK treatments induced
407 higher, although statistically non-significant, activities in many C and N
408 acquiring enzymes compared to N alone (Figures 6&7). The exceptions
409 to this were the suppression of β -xylosidase and β -glucuronidase
410 activities in *C. vulgaris* roots under $\text{NH}_4\text{Cl}+\text{PK}$ addition. Additionally,
411 there was a significant positive correlation between *C. vulgaris* ERM
412 intracellular hyphal frequency (ERM2) and β -glucuronidase activity ($r=$
413 0.66) and a statistically indicative positive correlation with β -glucosidase
414 activity ($r= 0.50$) (Figure 4).

415

416 3.4 Ericaceous Root Associated Fungi

417 Sanger sequencing of Direct PCR ITS amplicons from individual root
418 samples from both ericaceous species revealed several confirmed and
419 putative ERM/DSE fungal species, as well as a range of possible
420 endophytes, saprotrophs, and pathogens (Table 3). Fungal sequence
421 identifications based on Species Hypotheses (SH) ranged from 86% to
422 100% matches with reference sequences. Identifications of ascomycete
423 fungi likely inhabiting these ericaceous roots as ERM symbionts included
424 *Hyaloscypha hepaticicola* and *Hyaloscypha* sp., (names updated from
425 *Rhizoscyphus ericae* and *Meliniomyces* sp., respectively, according to
426 Fehrer et al. (2019)). Other members of the Leotiomycetes were also
427 identified, including unidentified Helotiales, unidentified
428 *Hyaloscyphaceae*, *Phialocephala sphaeroides*, and *Pseudogymnoascus*
429 sp. Furthermore, identified basidiomycetes which are capable of the
430 ERM lifestyle included members of the family *Serendipitaceae* (syn.
431 clade B Sebaciniales) and its member genus *Serendipita*. Interestingly,
432 members of the Helotiales were found only in roots of both hosts from
433 control, NH₄Cl or NH₄Cl+PK treatments while unidentified Pezizales
434 members were only detected in NaNO₃+PK treatments. Other
435 ericaceous root associated fungi which were possibly living saprotrophic
436 or pathotrophic lifestyles are presented in Table 3.

437 Table 3. Fungal identifications from Sanger sequencing of Direct PCR ITS amplicons from *C. vulgaris* and *E. tetralix* roots. % ID values indicate range of similarity
 438 with reference sequences according to Unite database. Function assignment according to FUNGuild analysis. Sequences are from 38 samples with ITS
 439 amplicons >200 bp as required by Unite Gen Bank, see Supplementary File 1 for all 57 FASTA sequences. Sources of sequences listed by treatments and host
 440 species indicated with Cv and Et, numbers in brackets indicate the number of replicate plots with the same sequence, subscripts c=Control, 1=NaNO₃,
 441 2=NaNO₃+PK, 3=NH₄Cl, 4=NH₄Cl+PK.

Phylum	Class	Order	Family	Species	% ID	Function	Reference Seq(s) SH	Source	
Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	<i>Archaeorhizomycetaceae</i>	<i>Archaeorhizomyces sp.</i>	97.58	Sapro	KT768305 SH180923.07FU	Cv _c	
	Dothideomycetes	Capnodiales	<i>Mycosphaerellaceae</i>	<i>Acrodontium crateriforme</i>	95.16	Patho-Sapro	KX287271 SH214154.07FU	Cv ₄	
			<i>Cladosporiaceae</i>	<i>Cladosporium sp.</i>	99.77	Patho-Sapro-Symbio	KX459429 SH212842.07FU	Cv ₁	
	Leotiomycetes	Helotiales	<i>Hyaloscyphaceae</i>	<i>Hyaloscypha hepaticicola*</i>	99.07	Patho-Sapro-Symbio	FM172802 SH181107.07FU	Cv ₃	
				<i>Hyaloscypha sp.*</i>	99.18- 99.59	Sapro-Symbio	FM997935 SH025067.07FU DQ309217 SH214267.07FU	Cv _c ; Cv/Et ₃ ;Cv ₄	
				Unidentified	99.79	Sapro	HF947840 SH004619.07FU	Et _c	
			<i>Vibrisseaceae</i>	<i>Phialocephala sphaeroides</i>	91.06	Symbio	KC480051 SH204990.07FU	Et ₃	
			Unidentified	Unidentified	88.89- 99.61	-	HF947859 SH218310.07FU AF252840 SH211416.07FU HF947861 SH197071.07FU AY627806 SH201639.07FU	Cv _c ; Cv/Et(2) ₃	
			Rhytismatales	Unidentified	Unidentified	92.00- 95.71	-	AF149078 SH183994.07FU DQ309240 SH143881.07FU	Cv/Et _c ;Cv ₁
			Thelebolales	<i>Pseudeurotiaceae</i>	<i>Pseudogymnoascus sp.</i>	93.89	Patho-Sapro-Symbio	KP902680 SH183329.07FU	Cv ₂
	Pezizomycetes	Pezizales	Unidentified	Unidentified	95.94- 100	-	JQ347011 SH203769.07FU	Cv(2)/ Et(2) ₂	
	Sordariomycetes	Microascales	<i>Halosphaeriaceae</i>	Unidentified	94.64	Sapro	FJ524322 SH211311.07FU	Et ₂	
	Taphrinomycetes	Taphrinales	<i>Taphrinaceae</i>	<i>Taphrina tormentillae</i>	99.14	Patho	KX516468 SH200748.07FU	Et _c	
	incertae sedis	incertae sedis	incertae sedis	<i>Xenochalara sp.</i>	96.04	Sapro	HM230882 SH202721.07FU	Et ₄	
	Basidiomycota	Agaricomycetes	Agaricales	<i>Entolomataceae</i>	<i>Entoloma cetratum</i>	97.14	Patho-Sapro-Symbio	KC898450 SH185814.07FU	Cv ₂
<i>Tricholomataceae</i>				Unidentified	93.82	Patho-Symbio	KY701558 NA	Cv ₃	
<i>Strophariaceae</i>				<i>Pholiota sp.</i>	86.47	Sapro	HQ533029 SH219745.07FU	Et ₂	
		Cantharellales	<i>Ceratobasidiaceae</i>	<i>Ceratobasidium sp.</i>	99.49	Patho-Sapro-Symbio	JN569114 SH220624.07FU	Cv ₃	
		Jaapiales	<i>Vibrisseaceae</i>	<i>Jaapia ochroleuca</i>	99.31	Sapro	UDB031153 SH190037.07FU	Et ₃	
		Sebacinales	<i>Serendipitaceae</i> (Clade B Sebacinales)	<i>Serendipita sp.</i>	98.25- 100	Symbio	GQ907110 SH003898.07FU HF947895 SH201953.07FU DQ309211 SH179088.07FU DQ309149 SH180008.07FU	Et _c ; Cv(2) ₁ ; Et(2) ₃	
		Unidentified		94.35- 100	-	HF947869/HF947915 /DQ309208 SH179085.07FU	Cv(2) ₂ ; Cv/Et ₄		
			<i>Sebacinaceae</i>	Unidentified	99.66	Symbio	HQ154421 SH199330.07FU	Cv _c ;Et ₂	
		Thelephorales	<i>Thelephoraceae</i>	<i>Thelephora terrestris</i>	98.72	Symbio	KX438350 SH184510.07FU	Et ₃	
Mortierellomycota		Mortierellomycetes	Mortierellales	<i>Mortierellaceae</i>	<i>Mortierella humilis</i>	100	Sapro-Symbio	KM504403 SH196779.07FU	Cv/Et ₃
			<i>Mortierella parvispora</i>	99.83	Sapro-Symbio	FN565295 SH193938.07FU	Et ₄		
Rozellomycota	unidentified	Unidentified	Unidentified	Unidentified	98.23	-	KF297176 SH204524.07FU	Et ₃	

442 *Sequences identified as *Rhizoscyphus ericae* and *Meliniomyces sp.* updated to as *Hyaloscyphus hepaticicola* and *Hyaloscyphus sp.*, respectively, according to Fehrer et al. (2019)

443 3.5 Ericaceous Root Enzyme Activity Profiles

444 When fungal sequence identifications were linked to the enzymatic
445 activities of their respective root samples, potential species related
446 enzyme activity patterns emerged (Table 3, Figure 8). *C. vulgaris* root
447 samples from NaNO₃, NaNO₃+PK, and NH₄Cl+PK treatments and hosting
448 *Serendipitaceae* (Clade-B Sebaciniales) or *Serendipita* sp. produced the
449 highest detected leucine aminopeptidase activities. A *C. vulgaris* root
450 sample in the NH₄Cl+PK treatment and hosting *Hyaloscypha* sp. was
451 highly active in β-xylosidase, N-acetylglucosaminidase, and β-
452 glucosidase, while samples hosting *Hyaloscypha* sp. in control and NH₄Cl
453 treatments were ca. 50-75 % less active for the same enzymes.
454 Additionally, a *C. vulgaris* root sample in the NH₄Cl treatment
455 simultaneously hosting *Hyaloscypha hepaticicola* and *Ceratobasidium*
456 sp. was one of the most active samples, across all enzymes.
457
458 Interestingly, a root sample from *C. vulgaris* in the NaNO₃+PK treatment
459 was linked to a fungal identification of *Pseudogymnoascus* sp., which
460 produced some of the highest activities for all enzymes in this
461 treatment, except leucine aminopeptidase. A sample of *C. vulgaris* root
462 from the NH₄Cl+PK treatment which hosted the species *Acrodontium*
463 *crateriforme* indicated activities of cellobiohydrolase, N-
464 acetylglucosaminidase, and β-glucosidase several times higher than any
465 other samples measured. In the control treatment, *C. vulgaris* root
466 samples hosting a member of the Rhytismatales showed the highest

467 activity for leucine aminopeptidase and acid phosphatase while *E.*
468 *tetralix* samples hosting Rhytismatales showed the highest activities for
469 all enzymes in that treatment, except leucine aminopeptidase.

470

471 **4 Discussion**

472 The increases in overall fungal colonization for both *Calluna*
473 *vulgaris* and *Erica tetralix* under nutrient addition were unexpected and
474 the unchanged frequency of ERM intracellular coiling showed that long-
475 term N and NPK addition did not reduce mycorrhizal colonization. This
476 suggests that the ericaceous host plants are unable to restrict fungal
477 colonization of their roots, despite access to excess inorganic N and P.
478 Alternatively, unchanged mycorrhizal colonization rates may indicate
479 that the fungi provide benefits to the plant beyond N and P.

480

481 The observed reduction of *Sphagnum* abundance and the decreased
482 frequency of DSE associated with ericaceous roots may be linked. DSE
483 fungi have been shown to be a common occurrence in submerged
484 aquatic plants (Kohout et al., 2012) and are capable of propagating and
485 existing as saprobes of moss gametophytes (Day and Currah, 2011). This
486 capability and their prevalence in aquatic plants suggest that DSE fungi
487 are well adapted to periodic waterlogged conditions in peatlands,
488 perhaps providing a niche during the annual senescence of their
489 ericaceous hosts. A similar relationship between ERM fungi and
490 liverworts, common species in peatlands, may also provide a niche

491 during host senescence (Kowal et al., 2018, 2015). As the loss of
492 *Sphagnum* species is one of the most obvious effects of nutrient
493 addition treatments (Bubier et al., 2007; Levy et al., 2019), this may
494 remove an important ecosystem niche for DSE fungi. Our finding of
495 positive correlation between *E. tetralix* root surface DSE colonization
496 frequency and *Sphagnum* abundance supports this possibility.

497

498 The significantly increased frequencies of ERM hyphal morphotypes and
499 decreased DSE hyphal morphotypes in ericaceous roots strongly
500 suggests that long-term nutrient addition resulted in an altered fungal
501 community. Furthermore, the different forms of N addition may have
502 had selective effects on the fungal community as we identified different
503 root associated Helotiales members only in controls, NH_4Cl or $\text{NH}_4\text{Cl}+\text{PK}$
504 treatments and members of the Pezizales only in NaNO_3+PK treatments.
505 The Helotiales may prefer NH_4^+ as a substrate compared to NO_3^- , as
506 experimental evidence has shown for *Hyaloscypha hepaticicola* (Cairney
507 et al., 2000). The presence of the Pezizales in only NaNO_3+PK
508 treatments may imply they prefer this N source, though their
509 mycorrhizal status is unclear it has been suggested for some families by
510 Hobbie et al. (2001).

511

512 Sequence identification of ITS amplicons from root samples of both
513 ericaceous species revealed several ERM and DSE species commonly
514 found to associate with ericaceous roots, as well as common peatland

515 saprotrophs and pathogens (Sietiö et al., 2018; Thormann, 2006;
516 Thormann and Rice, 2007). Both ericaceous species shared several
517 family and genus level groups, indicating a common symbiont
518 community among the ericaceous hosts. This is in line with the findings
519 of Kjølner et al. (2010) who showed that several ericaceous species in a
520 subarctic mire shared fungal communities when in close proximity.
521 While ERM symbionts are generally ascomycetes, recent studies have
522 found that the basidiomycete fungi *Serendipitaceae* (Clade-B
523 Sebaciales) are common in ericaceous roots and capable of forming
524 mycorrhizal structures (Brundrett and Tedersoo, 2018; Vohník et al.,
525 2016; Weiß et al., 2016), and potentially capable of utilizing
526 photosynthetic C (Sietiö et al., 2018). Our identifications of members of
527 the *Serendipitaceae* and *Serendipita* sp. in both ericaceous hosts
528 support their likely role as root symbionts. Interestingly, in *C. vulgaris*
529 roots from the NaNO₃+PK treatment we found a potential ERM
530 symbiont, *Pseudogymnoascus* sp., which is a genus that may form ERM
531 associations, as shown between *Pseudogymnoascus roseus* and
532 *Vaccinium angustifolium* (Dalpé, 1989).
533
534 Our findings on the varying effects of N addition on root surface
535 enzymatic activities in the two ericaceous species, *C. vulgaris* and *E.*
536 *tetralix*, indicate that they may have functionally different root
537 symbionts and decomposition potentials. In NPK treatments both
538 ericaceous plants displayed highly suppressed acid phosphatase activity

539 as the roots and root associated fungi did not need to access organic P
540 sources. Conversely, N treatments increased acid phosphatase activity,
541 reflecting the colimitation of N and P found in peatlands (Pinsonneault
542 et al., 2016; Wang et al., 2015) and confirming that ericoid mycorrhizal
543 root enzymatic activities reflect nutrient limitations. Almost every
544 enzyme activity increased with additional P compared to either form of
545 N alone, while NaNO_3 addition was found to generally decrease enzyme
546 activities for *C. vulgaris*. Additionally, both forms of N addition led to
547 reductions in chitinase activity, which is similar to the findings of
548 Bragazza et al. (2006), who suggested that this indicates an alleviation
549 of N limitation.

550

551 In *C. vulgaris* roots β -xylosidase, β -glucuronidase, and β -glucosidase
552 activities under NH_4Cl addition were comparable to or higher than their
553 activities in controls and NaNO_3 +PK treatments, suggesting that NH_4Cl
554 promotes overall decomposition activity, as these enzymes primarily
555 degrade plant cell wall components (Dunn et al., 2014). This may reflect
556 a reduction in N limitation for the *C. vulgaris* mycorrhizal symbiont
557 identified as *Hyaloscypha hepaticicola*, as experimental *in vitro* data has
558 shown that this species may preferentially utilize NH_4^+ as a source of N
559 compared to organic sources (Cairney et al., 2000). Additionally, this is
560 supported by the colonization morphotype data showing that NH_4Cl
561 addition induced the largest significant increase in intracellular ERM
562 hyphae in *C. vulgaris*, implying increases in fungal biomass and

563 therefore higher decomposition potential. Furthermore, the sequence
564 identification of *H. hepaticicola* in a *C. vulgaris* root sample from the
565 NH₄Cl treatment was linked to relatively higher enzymatic activities
566 compared to other root samples in the same treatment. *Calluna vulgaris*
567 roots hosting *Hyaloscypha* sp. displayed higher enzymatic activity under
568 nutrient addition than in control conditions, indicating a response to
569 increased N or NPK availability.

570

571 Our results demonstrate that it is necessary for studies of mycorrhizal
572 fungi to include measurements of enzyme activities in natural
573 conditions in order to more precisely estimate their roles in nutrient
574 cycles. Though there is extensive research on the enzymatic activity of
575 mycorrhizal fungi in sterile systems, few studies have measured the
576 activity of mycorrhizal roots in their natural environment. While these *in*
577 *vitro* enzyme activities of mycorrhizal fungi are often interpreted as
578 their natural activities, work by Timonen and Sen (1998) showed that
579 enzyme expression levels in *Pinus sylvestris* mycorrhizal fungi are locally
580 regulated in the mycorrhizosphere, highlighting the variability in fungal
581 enzyme expression which is not apparent from *in vitro* studies.

582

583 We observed that long-term nutrient addition resulted in a reduction in
584 *C. vulgaris* abundance, potentially due to a reduction in competitive
585 fitness, leaving an ecosystem gap that was rapidly occupied by other
586 fast-growing species such as the non-mycorrhizal sedge *Eriophorum*

587 *vaginatum*, which is not reliant on symbionts for organic N uptake
588 (Chapin et al., 1993). The suppressive nature of NaNO₃ on *C. vulgaris*
589 root enzymatic activities, compared to *E. tetralix*, suggests that *C.*
590 *vulgaris* and its symbionts are more sensitive to NaNO₃ and its effects
591 on peat properties, such as pH. This is also reflected in the vegetation
592 abundance data for NaNO₃ where *C. vulgaris* abundance is reduced
593 while *E. tetralix* abundance increases. This sensitivity to NaNO₃ may put
594 *C. vulgaris* at a competitive disadvantage to other ericaceous species
595 during NO₃⁻ deposition.
596
597 Our findings of the cumulative effects of nutrient addition treatments at
598 Whim Bog on abundances of peatland vegetation are similar to those
599 detailed in Levy et al. (2019) which describes the decline of several plant
600 species and the increase of *E. vaginatum* as the major effects over the
601 entire timespan of the experimental site. The loss of *Sphagnum* may
602 also directly reduce the ability of ericaceous species to uptake nutrients,
603 as the upper moss layer is heavily inhabited by ericaceous roots,
604 forming a thick layer which receives nutrients from litter and the
605 atmosphere before it reaches the lower layers (Read et al., 2004). As the
606 living *Sphagnum* layer is lost and forms bare, decaying peat, it collapses
607 and becomes more submerged and anoxic, becoming an environment
608 that ericaceous roots are less likely to inhabit. This loss of aerobic
609 substrate for ericaceous species to inhabit and uptake nutrients from
610 may be an underlying cause of the observed reduction in *C. vulgaris*

611 abundance. The loss of the moss layer may also lead to the subsidence
612 of peat, as has been observed by Juutinen et al. (2018) to be a result of
613 nutrient addition at another long-term nutrient addition experiment,
614 Mer Bleue Bog, located in Ontario, Canada. This subsidence was
615 indicated by our water table depth measurements, as they were made
616 relative to the moss surface and the treatments with the highest water
617 table values, NaNO_3 and NaNO_3+PK , also showed the largest reductions
618 in moss abundance. Furthermore, the effects of the treatments on peat
619 pH should not be overlooked as a significant source of variation. Long-
620 term alteration of pH is likely directly linked to the observed differences
621 in fungal colonization patterns as well as root enzymatic activity.
622
623 Loss of ericaceous vegetation and *Sphagnum* are key examples of the
624 risks posed by anthropogenic N and P deposition. Current research has
625 lacked a clear picture of how ericaceous root associated fungi, ERM and
626 DSE, are involved in these processes. Our findings indicate that altered
627 nutrient limitations shift root associated fungal diversity and
628 morphology, with direct effects on enzyme activity and thereby
629 decomposition potential. The losses of *C. vulgaris* and *Sphagnum* due to
630 nutrient addition and the subsequent reduction in DSE colonization
631 rates may have additional consequences. Dark septate endophytes are
632 by nature heavily melanized and may contribute a significant source of
633 recalcitrant C (Fernandez and Koide, 2014). Subsequently, the potential
634 loss of recalcitrant fungal biomass may lead to lower peatland C

635 sequestration. As suggested by Averill et al. (2014) and Orwin et al.
636 (2011), ERM symbionts may be responsible for limiting the
637 decomposition potential of free-living saprotrophs and the microbial
638 community by increasing N and P limitation in soil. Addition of inorganic
639 N and P may upset this limitation, leading to increased decomposition
640 which releases C limitation for the more efficient saprotrophs, which in
641 turn outcompete the mycorrhizal fungi, thereby limiting ericaceous
642 nutrient access. Further research is necessary to determine the
643 potential long-term risks of N and P deposition and the role of peatland
644 mycorrhizal fungi in C sequestration.

645

646 4.1 Conclusions

647 The total frequency of fungal colonization at Whim Bog, Scotland, did
648 not decrease under nutrient addition treatments but instead tended to
649 increase by ca. 10% in both *Calluna vulgaris* and *Erica tetralix*, refuting
650 our hypothesis (1) which expected a reduction in fungal colonization
651 rates. The considerable increase in ERM hyphal frequency (ca. 20-30%)
652 in both host species was accompanied by a significant suppression of
653 DSE hyphal frequency (ca. 20-30%) under nutrient addition, indicating a
654 strong treatment effect on the root symbiont community. The altered
655 fungal morphotype frequencies and identified fungal species agree with
656 our hypothesis (2) of mycorrhizal diversity indicating nutrient addition
657 effects and suggests that altered nutrient availability is a selective
658 pressure upon the root associated fungal community. The enzymatic

659 activities of both ericaceous shrub roots and their associated fungi
660 strongly support our hypothesis (3) of mycorrhizal root enzyme
661 activities reflecting nutrient limitations.

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923 **Figure Captions**

924 Figure 1. Total observed ericoid mycorrhizal (ERM) and dark septate
925 endophyte (DSE) occurrence in *C. vulgaris* and *E. tetralix* roots as
926 determined by light microscopy and the magnified intersections
927 method. Error bars indicate ± 1 standard deviation, $n=3$.

928

929 Figure 2. Microscopically observed frequencies of potential ericoid
930 mycorrhizal morphotypes (ERM1-4) in *C. vulgaris* and *E. tetralix* roots.
931 Error bars indicate ± 1 standard deviation, $n=3$. Different superscript
932 letters indicate significant differences ($P<0.05$) compared with the other
933 treatments.

934

935 Figure 3. Microscopically observed frequencies of dark septate
936 endophyte morphotypes (DSE1-4) in *C. vulgaris* and *E. tetralix* roots.
937 Error bars indicate ± 1 standard deviation, $n=3$. Different superscript
938 letters indicate significant differences ($P<0.05$) compared with the other
939 treatments.

940

941 Figure 4. Correlation analysis of *Calluna vulgaris* root enzyme activities
942 (Leu: leucine aminopeptidase, Glr: β -glucuronidase, Xyl: β -xylosidase,
943 Cell: cellobiohydrolase, GlS: β -glucosidase, Nag: N-
944 acetylglucosaminidase, Pho: acid phosphatase), root associated fungal
945 morphotype categories ERM1-4 and DSE1-4 (Table 1), surface peat (0-
946 20cm) pH, plot water table depth (WT), *C. vulgaris* abundance (Cv), *Erica*

947 *tetralix* abundance (Et), *Eriophorum vaginatum* abundance (Et), other
948 vascular spp. abundance (OV), *Sphagnum* abundance (Sph), and other
949 moss spp. abundance (OM). Statistically significant and indicative
950 correlation values are indicated by asterisks (** for $p < 0.01$, * for $p < 0.05$,
951 and + for $p < 0.1$) values, with a greyscale ranging from lighter shades
952 indicating positive correlation coefficients to darker indicating negative
953 correlation coefficients, $n=15$.

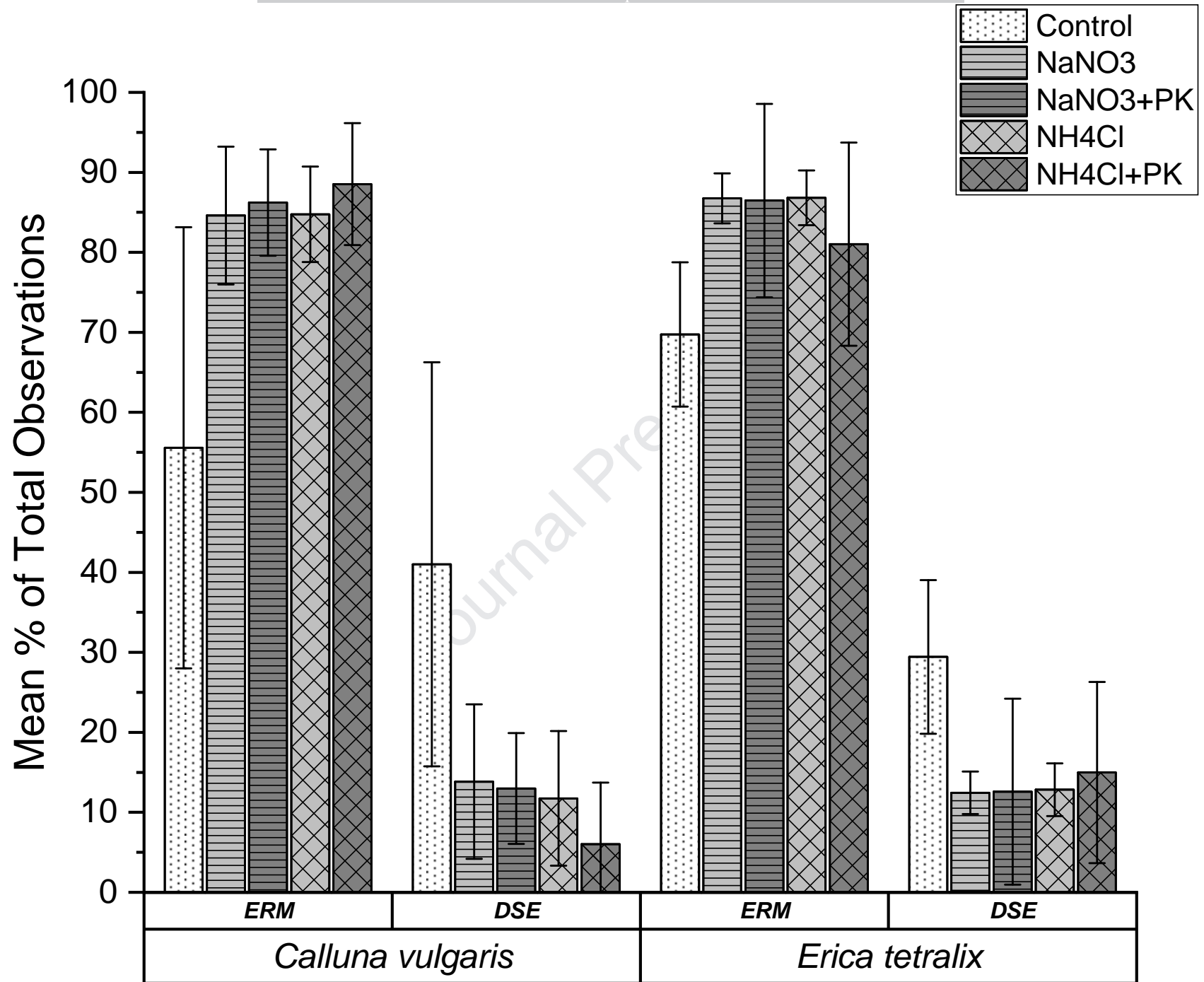
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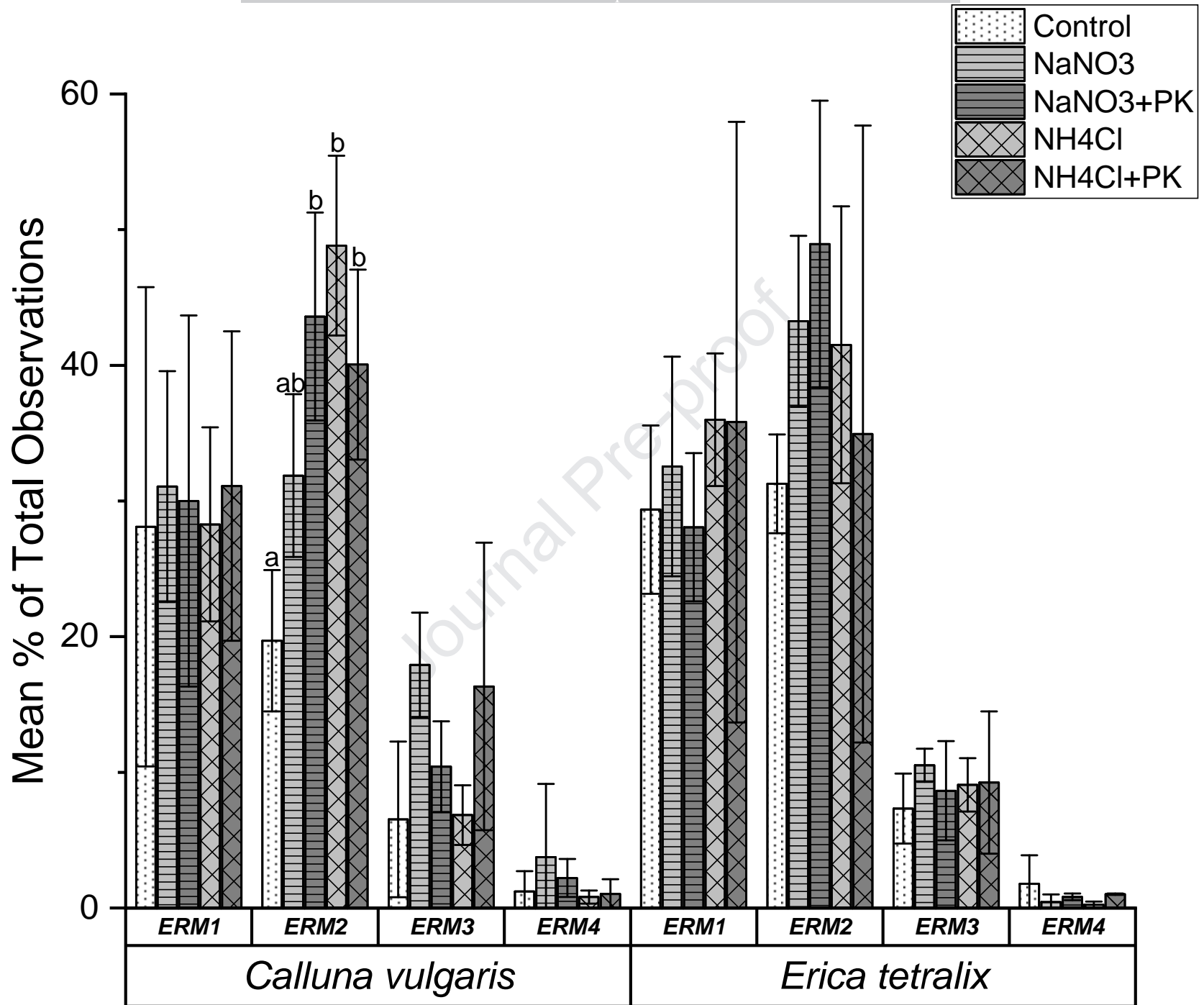
955 Figure 5. Correlation analysis of *Erica tetralix* root enzyme activities
956 (Leu: leucine aminopeptidase, Glr: β -glucuronidase, Xyl: β -xylosidase,
957 Cell: cellobiohydrolase, Gls: β -glucosidase, Nag: N-
958 acetylglucosaminidase, Pho: acid phosphatase), root associated fungal
959 morphotype categories ERM1-4 and DSE1-4 (Table 1), surface peat (0-
960 20cm) pH, plot water table depth (WT), *Calluna vulgaris* abundance
961 (Cv), *E. tetralix* abundance (Et), *Eriophorum vaginatum* abundance (Et),
962 other vascular spp. abundance (OV), *Sphagnum* abundance (Sph), and
963 other moss spp. abundance (OM). Statistically significant and indicative
964 correlation values are indicated by asterisks (** for $p < 0.01$, * for $p < 0.05$,
965 and + for $p < 0.1$) values, with a greyscale ranging from lighter shades
966 indicating positive correlation coefficients to darker indicating negative
967 correlation coefficients, $n=15$.

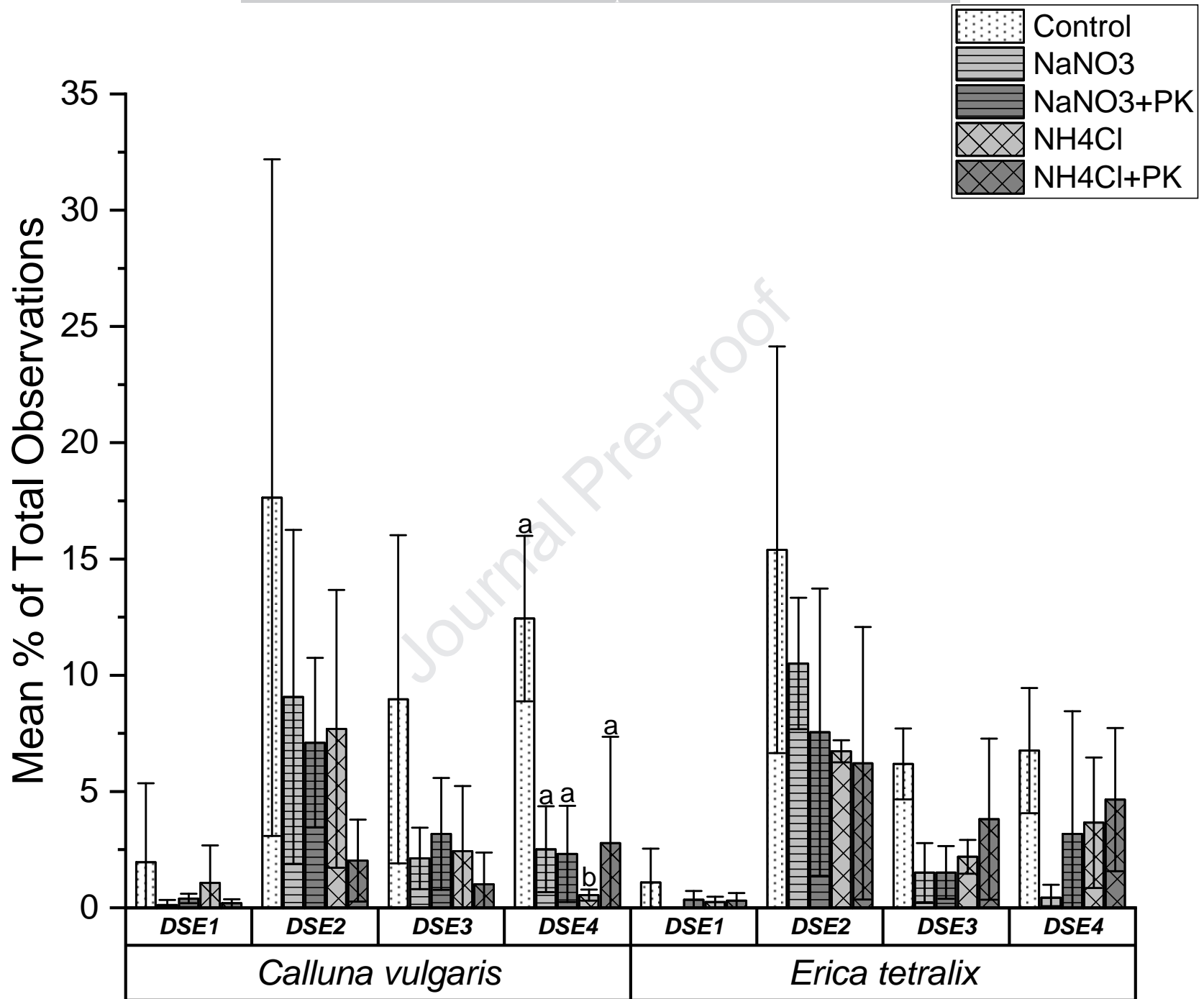
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969 Figure 6. *C. vulgaris* and *E. tetralix* root surface enzyme activities
970 according to treatment means (Leu: leucine aminopeptidase, Xyl: β -

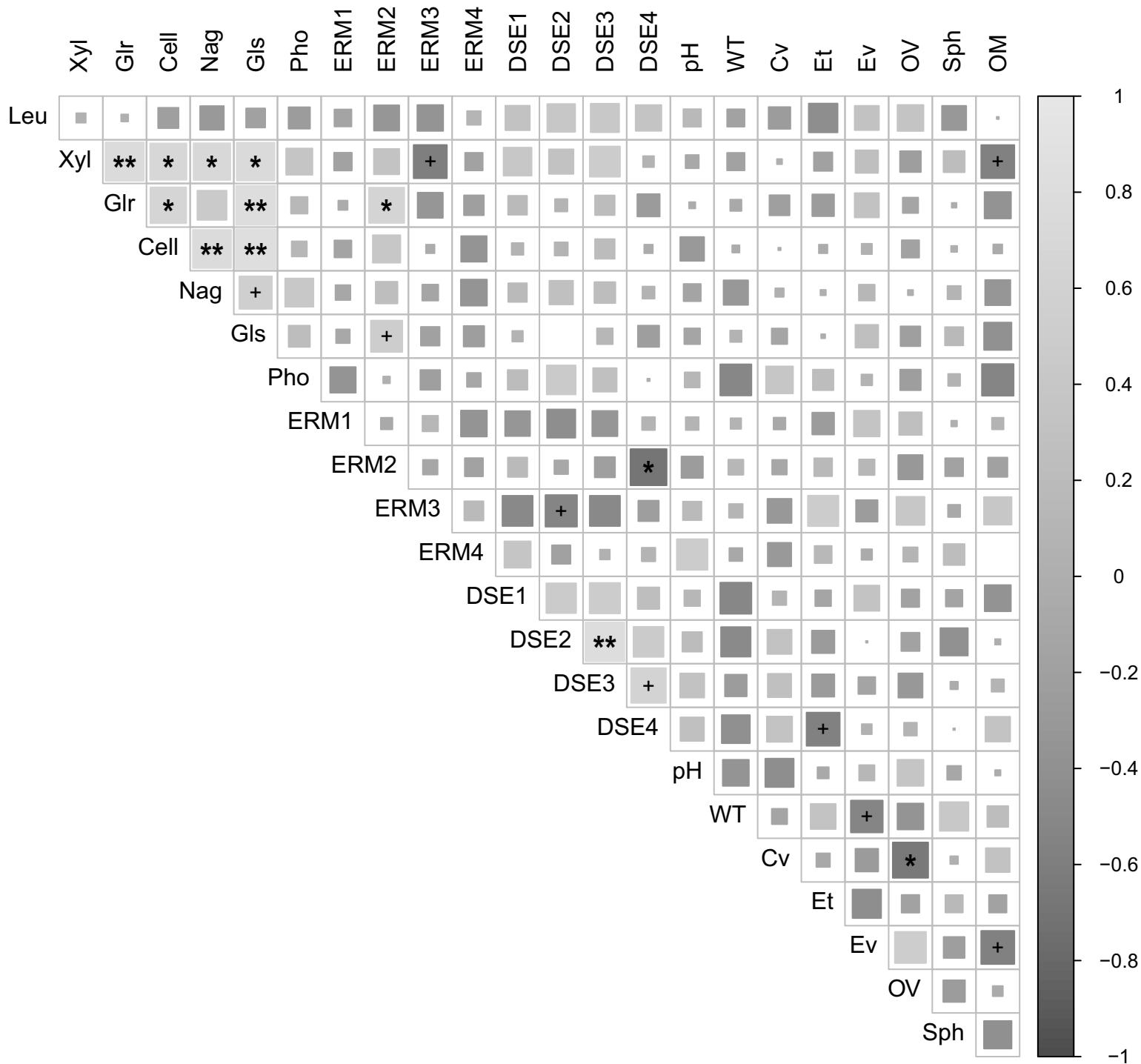
971 xylosidase, Glr: β -glucuronidase, Cell: cellobiohydrolase). Error bars
972 indicate ± 1 standard deviation, n=3.
973
974 Figure 7. *C. vulgaris* and *E. tetralix* root surface enzyme activities
975 according to treatment means (Nag: N-acetylglucosaminidase, GlS: β -
976 glucosidase, Pho: acid phosphatase). Error bars indicate ± 1 standard
977 deviation, n=3. Letters above error bars indicate statistically significant
978 differences.
979
980 Figure 8. Ericaceous root enzyme activities (Leu: leucine
981 aminopeptidase, Glr: β -glucuronidase, Xyl: β -xylosidase, Cell:
982 cellobiohydrolase, GlS: β -glucosidase, Nag: N-acetylglucosaminidase,
983 Pho: acid phosphatase) for individual root pieces with identified fungal
984 sequences, as well as the mean activities for root samples without
985 sequencing (Black X Symbol, $20 < n < 25$, per treatment). Error bars
986 indicate ± 1 standard deviation of the samples without sequencing. Not
987 shown; *Acrodontium crateriforme*: Xyl=0.61, Nag=7.5, (*C. vulgaris*,
988 $\text{NH}_4\text{Cl}+\text{PK}$). *Pseudogymnoascus* sp.: Cell=0.87, (*C. vulgaris*, NaNO_3+PK).

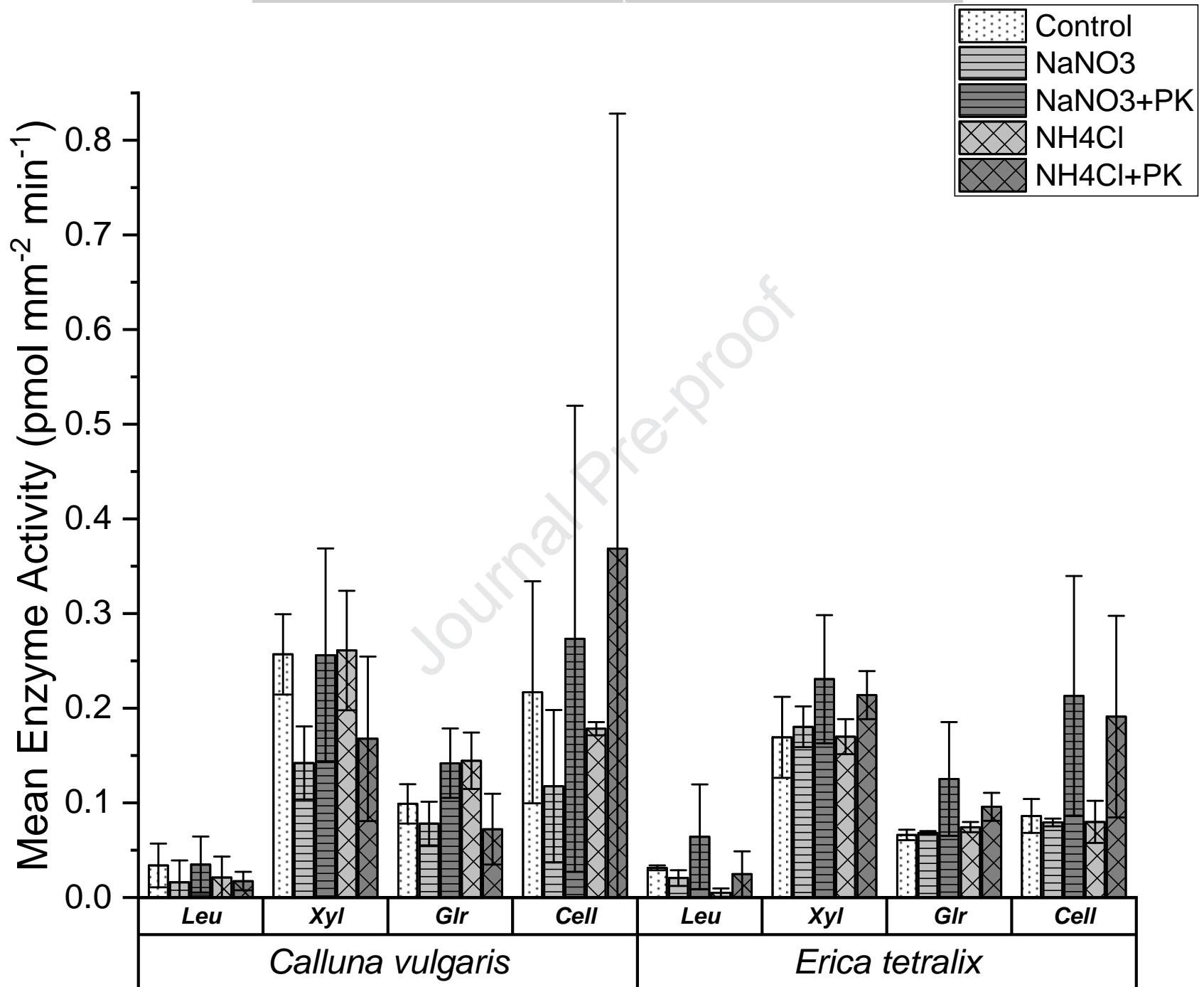


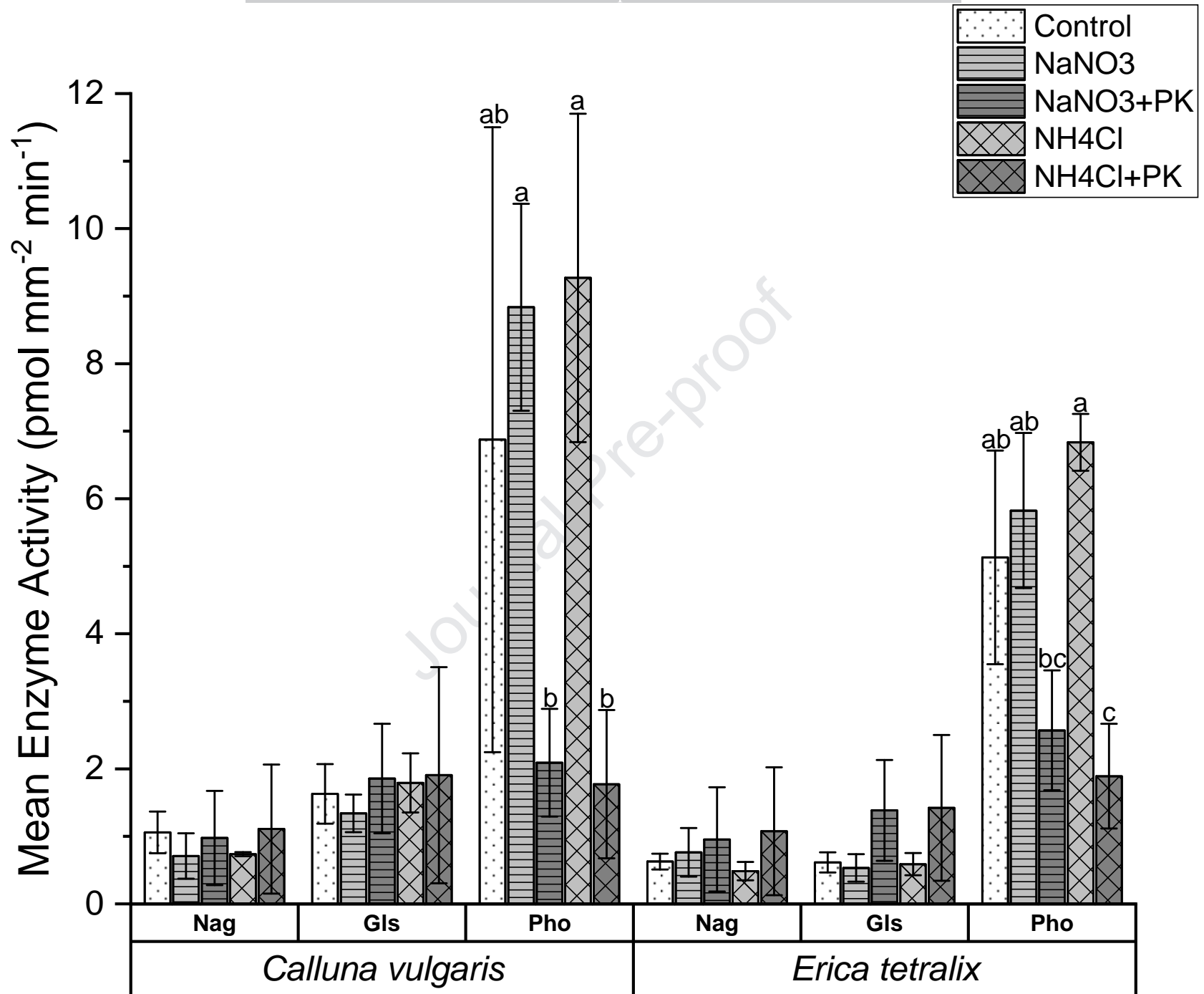


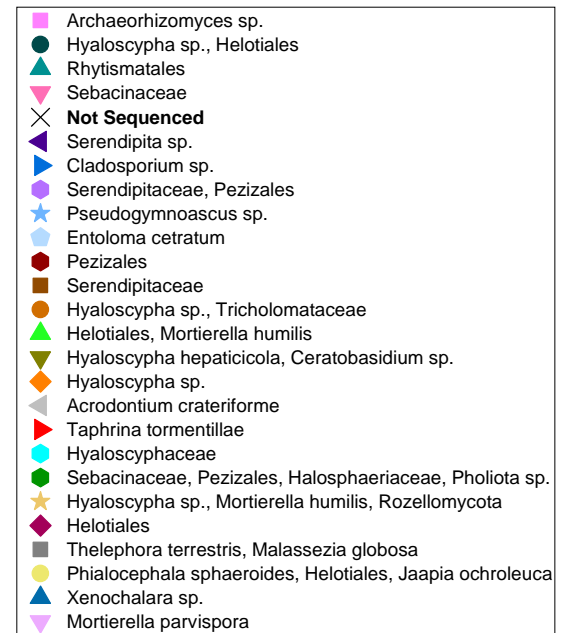
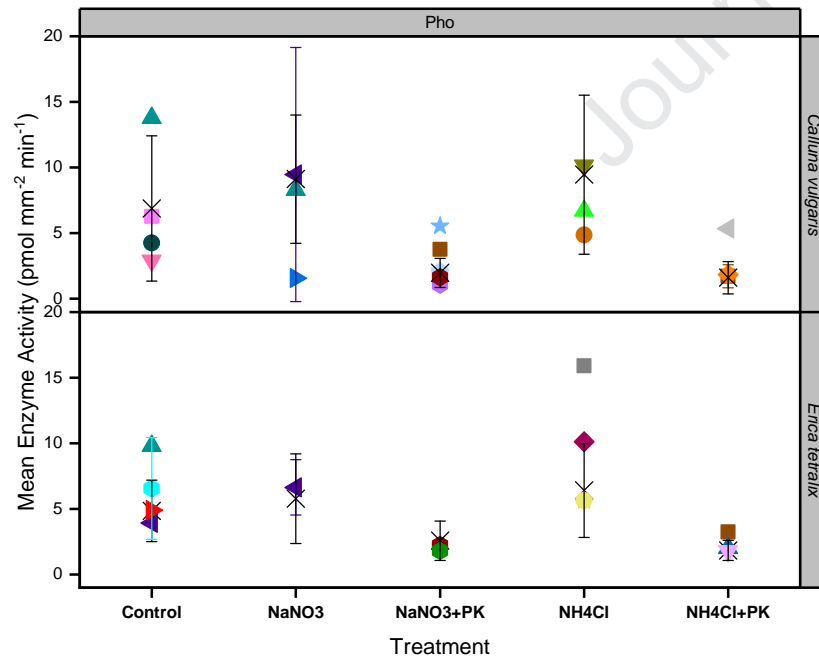
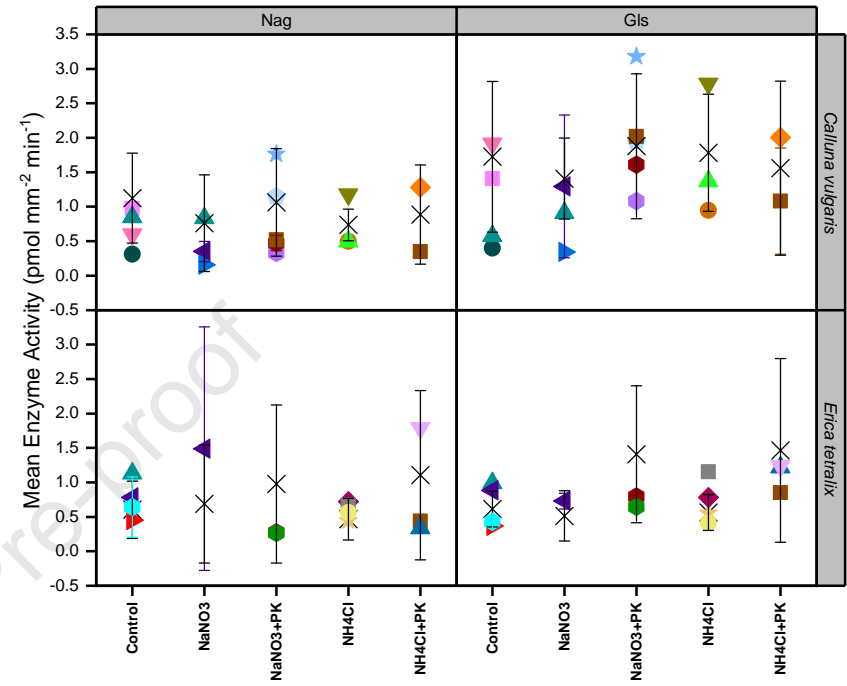
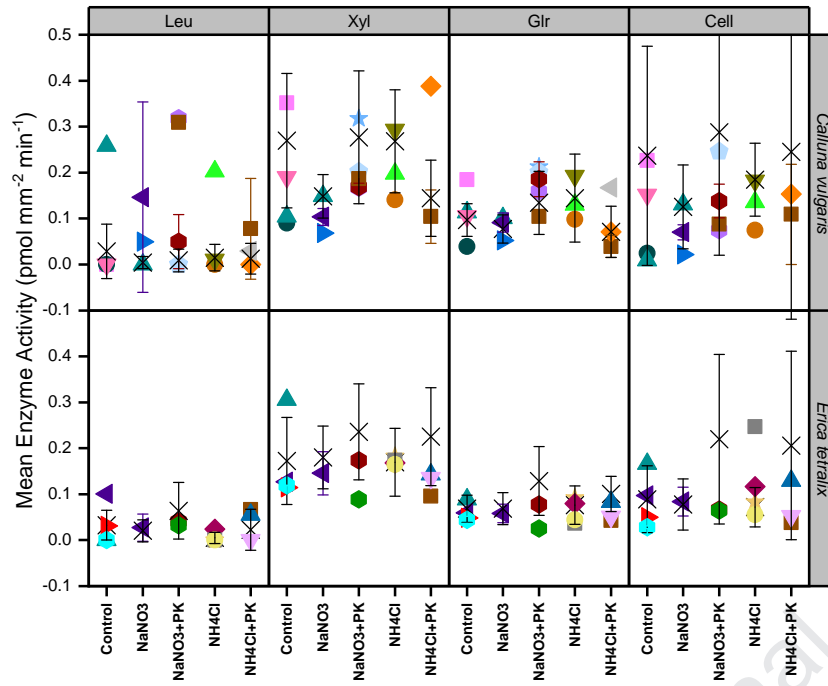


Calluna vulgaris Correlations









- Long-term effects of N/NP on ericaceous root fungal colonization & enzymatic activity
- ERM intracellular colonization unchanged, ERM hyphae increased & DSE hyphae decreased
- Loss of recalcitrant fungal biomass in melanized DSE may reduce peatland C sink
- Nitrate reduced *C. vulgaris* root enzyme activity, suggests host/symbiont sensitivity

Journal Pre-proof

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: