1   **Direct and indirect effects of land use on bryophytes in grasslands**

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Abstract

Land-use intensification is the major threat for biodiversity in agricultural grasslands, and

fertilization has been suggested as the most important driver. A common explanation for the

decline of bryophyte diversity with higher land-use intensity is an indirect negative effect via

the increase in vascular plant productivity, which reduces light levels for bryophytes.

However, direct negative effects of land-use intensification may also be important. Here, we

disentangle direct and vascular plant biomass mediated indirect effects of land use on

bryophytes. We analyzed two complementary datasets from agricultural grasslands, an

observational study across 144 differently managed grasslands in Germany and an

experimental fertilization and irrigation study of eleven grasslands in the Swiss Alps. We

found that bryophyte richness and cover strongly declined with land-use intensity and in

particular with fertilization. However, structural equation modelling revealed that although

both direct and indirect effects were important, the direct negative effect of fertilization was

even stronger than the indirect effect mediated by increased plant biomass. Thus, our results

challenge the widespread view that the negative effects of fertilization are mostly indirect and

mediated via increased light competition with vascular plants. Our study shows that land use

intensification reduces bryophyte diversity through several different mechanisms. Therefore,

only low-intensity management with limited fertilizer inputs will allow the maintenance of

bryophyte-rich grasslands.

1. Introduction

Extensively managed grasslands harbor a high diversity of many different taxa (Allan et al., 2014). However, on the majority of agricultural grasslands this diversity is threatened by land-use intensification (e.g., Kleijn et al., 2008; Allan et al., 2014; Gossner et al., 2016). With the aim to increase yield, semi-natural grasslands often receive large amounts of organic or inorganic fertilizer, often in combination with irrigation in drier regions. This results in long-term changes in species composition and in biodiversity loss (Humbert et al., 2016; Melts et al., 2018). In addition, these productive grasslands are mown more frequently or are grazed at higher stocking densities than in the past (Blüthgen et al., 2012). While these effects of land-use intensification on the diversity of vascular plants have been well studied (e.g., Kleijn et al., 2008; Socher et al., 2012), bryophytes have only rarely been considered. However, bryophytes a group including mosses, liverworts and hornworts, are often abundant in grasslands, where they constitute a substantial part of the total grassland plant diversity (Dengler et al., 2006, 2016) and contribute to several important ecosystem processes such as C and N cycles (Turetsky, 2003). As diversity, but also the abundance, of multiple taxa, including locally rare species, is important to maintain ecosystem functions (e.g. Soliveres et al., 2016a, 2016b), the loss of bryophyte diversity and abundance could lead to reduced ecosystem functioning in grasslands. Moreover, bryophyte diversity is a very good indicator of the overall diversity of grasslands (multidiversity), and the diversities of many individual plant and animal taxa (Manning et al., 2015). Understanding land-use effects on bryophytes is therefore important to better preserve this important group and to maintain basic ecosystem functions.

A number of observational and experimental studies have shown a negative relationship between bryophyte richness and vascular plant cover or grassland productivity, and that fertilization is one of the main drivers reducing bryophyte species richness and cover in

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grasslands (Jäppinen and Hotanen, 1990; Carroll et al., 2000; Bergamini and Pauli, 2001;
Aude and Ejrnæs, 2005; Bobbink et al., 2010; Verhoeven et al., 2011; Müller et al., 2012;
Boch et al., 2015; van Klink et al., 2017). Most of these studies have assumed that the main
mechanism driving the decline in bryophytes is an increase in vascular plant biomass, which
reduces light levels for low growing bryophytes. Both experimental (Hautier et al., 2009;
DeMalach and Kadmon, 2017; DeMalach et al., 2017) and observational studies (Grace et al.,
2016) have shown that an increase in light competition is the major driver of reduced plant
diversity at high productivity. However, a loss of resource niches could also contribute to
reducing plant diversity in fertilized conditions (reduced niche dimensionality hypothesis:
Harpole and Tilman, 2007; Harpole et al., 2017). Fertilization may also reduce plant diversity
via other mechanisms, such as toxicity and acidification (Bobbink et al., 2010) and these
direct negative fertilizer effects (in particular by ammonia) may also be important for
bryophytes (Jäppinen and Hotanen, 1990; Carroll et al., 2000; Krupa, 2003; Pearce et al.,
2003; Paulissen et al., 2004; Du et al., 2014; Andersen et al., 2016; Sun et al., 2017).
Moreover, fertilization effects depend on the physicochemical environment and may interact
with other components of land use. For instance, in drier regions fertilization requires
increased levels of irrigation to be effective, which may in turn also directly affect bryophyte
diversity (Mamolos et al., 2005). The relative importance of these direct fertilization effects,
mediated by changes in soil chemistry, compared to indirect effects mediated through changes
in plant productivity and light levels is not known.

Traditional land-use management, such as extensive mowing or grazing, is important for
maintaining semi-natural temperate grasslands and their diversity because it prevents shrub
encroachment and increase light levels for subordinate plant species (Pykälä, 2005; Hejcman
et al., 2013; Borer et al., 2014). However, increased mowing frequency – which generally
occurs together with greater fertilizer inputs – leads to a homogenous sward and can reduce

103 grassland diversity, including bryophyte diversity (e.g. Müller et al., 2012; Allan et al., 2014).

104 The effect of grazers is even more complex, as grazing removes biomass but also results in

105 trampling and the deposition of dung as well as urine. This creates habitat heterogeneity in

106 terms of unevenly deposited nutrients and sward cover and the trampling creates open soil

107 patches which provide microsites for seedling recruitment (Oldén et al., 2016). However,

108 similar to mowing, high grazing pressure can homogenize grasslands and reduce their overall

109 diversity (e.g. Pykälä, 2005; Allan et al., 2014). This means that grazing could have several

110 direct effects on bryophyte diversity, along with indirect effects mediated by changes in plant

111 biomass.

112 The aim of this study was to determine the importance of direct and indirect land-use effects

113 for bryophytes. For this, we used structural equation modelling (SEM; Shipley, 2002) which

114 is a powerful statistical tool in well-replicated comparative studies. Some studies have used

115 SEM to separate direct and indirect effects of fertilization, mowing and grazing on vascular

116 plant diversity (e.g., Socher et al., 2012), but this approach has only very rarely been used for

117 studies on bryophytes (but see Spitale et al., 2009). We fitted SEMs to two datasets: the first

118 is a large-scale observational dataset from the Biodiversity Exploratories project, which

119 includes 150 grasslands in three regions of Germany differing in land-use intensity. The

120 second is a dataset from the Swiss Alps, in which fertilization and irrigation were

121 experimentally manipulated at various intensity levels in semi-natural grasslands. We

122 hypothesized that increasing fertilization, and also irrigation, should increase the biomass

123 production of vascular plants, and thereby decrease bryophyte richness and cover indirectly

124 via increased light competition. We expected this indirect effect to be stronger than the direct

125 effects of fertilization. As increasing intensities of mowing and grazing cause frequent

126 disturbance and are only done on productive grassland, we expected them to reduce bryophyte

127 species richness and cover, even though plant biomass is removed.

128

2. Methods

2.1 Study sites, land use and vegetation

We used two complementary datasets to investigate land-use effects on bryophytes. The first

dataset (called the observational dataset, henceforth) contains observational data on bryophyte

species richness along a land-use intensity gradient in German grasslands (the German

Biodiversity Exploratories; Fischer et al., 2010). The second dataset (called the experimental

dataset, henceforth) contains experimental data on bryophyte species richness from a

replicated field experiment that tested the effects of modern fertilization and irrigation upon

plant and invertebrate communities of Swiss mountain hay meadows, with the objective to

define optimal trade-offs for sustainable grassland management (e.g., Andrey et al., 2016;

Lessard-Therrien et al., 2017). Combining these two datasets therefore allowed us to

generalize findings across a range of different grassland types and to assess i) how

intensification of land-use components affects bryophyte species richness and ii) how

important direct intensification effects on bryophyte species richness are, compared to plant

biomass-mediated indirect ones (see details below). Nomenclature of bryophytes follows

Koperski et al. (2000).

2.2 The observational dataset from the German Biodiversity Exploratories project

The Biodiversity Exploratories comprise 150 grassland sites situated in three regions of

Germany: the UNESCO Biosphere area Schwäbische Alb (Swabian Jura), situated in a low

mountain range in South-western Germany, the National Park Hainich and its surrounding

areas, situated in the hilly lands of Central Germany, and the UNESCO Biosphere Reserve

Schorfheide-Chorin, situated in the young glacial lowlands of North-eastern Germany. The

152 three regions differ in climate, geology and topography (Tab. 1). The gradients of land use

153 and species pools are typical for the variation found among large parts of temperate lowland

154 Europe. Plots were selected to differ strongly in land-use intensity, whilst minimizing

155 confounding with soil conditions or space (for details see Fischer et al., 2010; Blüthgen et al.,

156 2012).

157 In 144 of these grassland sites, we recorded the species richness of terricolous bryophytes

158 (species growing on soil) and estimated the percentage cover per species in 4 m × 4 m plots in

159 summer 2007 and 2008. Information on land-use intensity was obtained via questionnaires

160 sent to farmers and land owners. Our plots included meadows (mown one to four times per

161 year for hay or silage production), pastures grazed by livestock at different densities (sheep,

162 cattle or horses), or grasslands which were mown once per year and grazed by livestock at

163 different densities, the so-called mown pastures. Grazing regimes differ among livestock

164 types: sheep-grazed pastures are rotational or grazed by traditional shepherding (minimum is

165 two grazing days per year) but pastures grazed by cattle or horses are mainly permanent (up to

166 240 grazing days per year). Plots were either unfertilized, or fertilized to different extents

167 (Fischer et al., 2010; Boch et al., 2016a). We quantified land-use intensity (LUI) using an

168 integrated measure, which sums up the standardized intensities of fertilization (kilograms of

169 nitrogen per hectare per year), mowing (number of cuts per year) and grazing (duration and

170 type of grazing animals, converted to livestock units), calculated as:

171 $$LUI[i] = \sqrt{\frac{F[i]}{F_{mean}} + \frac{M[i]}{M_{mean}} + \frac{G[i]}{G_{mean}}}$$

172 where F_{mean}, M_{mean} and G_{mean} are mean values of all 50 plots of each study region (for details

173 see Blüthgen et al., 2012). For example, a very low LUI of 0.5 can be achieved through 30

174 days of grazing per ha by one cow, an intermediate LUI of 1.5 corresponds to a meadow

175 which is mown twice and receives 60 kg N per year, and a relatively high LUI of 3.0

8

176   corresponds to a meadow which is mown three times and receives 130 kg N per year (Boch et

177   al., 2016b).

178   Other studies compared the performance of the overall LUI (with equal weighting of

179   components) to models with grazing, fertilization and mowing fitted separately (where the

180   components can vary in the strength and direction of effect) and showed that LUI was a better

181   predictor of several diversity measures (including bryophyte diversity) than the individual

182   components, indicating its robustness and the validity of considering equal effects of the land

183   use components (Blüthgen et al., 2012; Allan et al., 2014).

184   We also sampled aboveground vascular plant biomass (plant biomass hereafter), to assess

185   annual grassland productivity, by clipping the vegetation at a height of 5 cm in eight 50 cm $\times$

186   50 cm subplots. These subplots were adjacent to the plots in which we recorded bryophytes.

187   In meadows, we sampled plant biomass at the same time as the first hay harvest by the farmer.

188   In pastures and mown pastures, we temporarily fenced our subplots to ensure that the

189   vegetation had not been grazed before plant biomass sampling. The plant biomass samples

190   were pooled, dried for 48 h at 80 °C and weighed immediately after drying.

191

192   *2.3 The experimental dataset from the Swiss mountain hay meadows*

193   The second, experimental dataset originates from the canton of Valais in southwestern

194   Switzerland and contains data from semi-natural grasslands differing markedly from the

195   German ones in terms of altitude and climate (Tab. 1). In 2010, eleven extensively managed

196   meadows were selected, which were at least 4000 m^2 in size, had received no or very low

197   levels of fertilizer (only solid manure), and/or were irrigated during droughts before the onset

198   of the experiment, and had been mown once a year for at least the past ten years.

199 In each meadow, six circular plots with a diameter of 20 m were established with at least 5 m

200 between plots as buffer zone. Then, six different management treatments were randomly

201 assigned to the six plots and applied consistently in each plot for five years. The treatments

202 were control, irrigation only (medium intensity), fertilization only (medium intensity), and a

203 combination of both irrigation and fertilization at three different intensity levels (low,

204 medium, high). In these, amounts varied from 1/3, through 2/3 to 3/3 of the quantity

205 theoretically needed to achieve maximum hay yield, under local conditions and a mowing

206 regime consisting of two hay harvests per year. From mid-May to the beginning of September

207 sprinkler irrigation was applied weekly with 10, 20 or 30 mm of water added, depending on

208 the intensity level of the irrigation treatment. The plots were not irrigated when >20 mm rain

209 had fallen in the previous week. Twice per year, in spring and after the first hay harvest, the

210 fertilized plots received a water-dissolved solution of organic dried manure NPK pellets

211 (MEOC SA, 1906 Charrat, Switzerland) and mineral potassium-sulphate (K_2SO_4),

212 corresponding to the standard-farm liquid manure (2.4 kg N, 2 kg P_2O_5, and 8 kg K_2O per m^3

213 of solution; according to Sinaj et al., 2009). The total amount of added fertilizer (kg N

214 $ha^{-1}year^{-1}$) depended on the theoretical local hay production potential, calculated from pre-

215 experimental hay yield and site altitude (for details see Appendix A in Andrey et al., 2016).

216 In July 2015, we sampled all terricolous bryophyte species and estimated the percentage cover

217 per species in a 2 m × 4 m subplot within each of the 66 treatment plots. In addition, the

218 productivity of each plot was quantified twice; once before each hay harvest by the farmer.

219 Productivity was assessed by clipping the vegetation at a height of 6 cm in two 1.6 m^2

220 rectangle subplots, adjacent to the plots in which we recorded bryophytes. Then, the two

221 samples were pooled, dried at 105°C for 72 h and weighed.

222

223 *2.4 Statistical analysis*

224   All statistical tests were performed using R, version 3.2.4 (R Core Team, 2016). We used

225   linear mixed-effect models (lme4 package; Bates et al., 2015) to test effects of land-use

226   components and land-use intensity (LUI; observational dataset), amount of added fertilizer

227   and amount of added water (experimental dataset) on plant biomass production and bryophyte

228   richness.

229   When analyzing the observational grassland dataset, we included region with three levels as a

230   random factor and the land-use components as fixed factors. However, disentangling the

231   relative effects of the land-use components on bryophyte richness, bryophyte cover and

232   vascular plant biomass was not possible as mowing and fertilization intensity were strongly

233   confounded (both factors increase plant biomass and reduce bryophyte species richness and

234   cover; results are not shown). This is because fertilized grasslands are always mown more

235   frequently. Therefore, we calculated a combined fertilization and mowing intensity measure,

236   summing up the standardized intensities of fertilization and mowing (see above for details on

237   LUI calculations; Tab. 2) and fitted it together with grazing intensity. In addition, we fitted a

238   separate model with the compound LUI as a fixed factor and region as a random factor. As

239   this analysis yielded qualitatively similar results (Tab. A.1), we do not discuss it further.

240   For the experimental dataset, we fitted study site (11 levels) as a random factor to correct for

241   differences among sites. We included irrigation (amount of added water; mm/week) and

242   fertilization (amount of added nitrogen; kg N ha^{-1}year^{-1}) as continuous fixed effects. We also

243   included altitude as a co-variate and further tested its interaction with fertilizer, to test whether

244   fertilizer had different effects at different altitudes. The interaction between fertilizer and

245   altitude was never significant, so we excluded it from the final analysis and we do not discuss

246   it further. Fitting the experimental treatments as a categorical fixed factor instead of the

247   continuous fixed effects of fertilization and irrigation yielded qualitatively similar results, so

248   we do not discuss the results of the categorical fixed factor analysis further.

249   We further used structural equation modelling (SEM) to evaluate the direct and indirect

250   effects of increased LUI, as well as increased intensity of the land-use components

251   (fertilization, mowing and grazing separately; observational dataset) and treatment effects

252   (fertilization and irrigation; experimental dataset) on bryophyte richness. We first developed

253   an *a priori* model based on the known effects and relationships among the drivers of

254   bryophyte diversity. To avoid large differences in the variances among the factors and to

255   improve model convergence, we standardized all variables to a mean of 0 and standard

256   deviation of 1. Moreover, we tested the bivariate relationships between all variables to ensure

257   that a linear model was appropriate.

258   In the observational dataset, we first corrected for regional differences by fitting a linear

259   model with region as a fixed factor to the species richness of bryophytes and the vascular

260   plant biomass. We then used residuals in subsequent analyses for estimating path coefficients,

261   using the lavaan package (Rosseel, 2012). As the land-use components fertilization, mowing

262   and grazing were already regionally standardized (see above), we used these values for further

263   analysis. As mowing (cutting frequency) and fertilization intensity were strongly confounded

264   (see explanation above) we introduced a composite variable into our model, which

265   summarises the combined effects of mowing and fertilization intensity. The use of composite

266   variables does not alter the SEM model but collapses the effects of conceptually related

267   variables into a single composite effect (Grace, 2006). As some variables were not perfectly

268   normally distributed, we confirmed the fit of the model using bootstrapping with 1000

269   iterations. We also tested the direct and indirect effect of the integrated measure of LUI on

270   bryophyte species richness in a separate analysis. As the analysis yielded qualitatively similar

271   results (Supplementary material Fig. A.1) but was less informative because the effect of

272   grazing is not separated from the combined fertilization and mowing effect, we do not discuss

273   the results further.

274   In the experimental dataset, we estimated path coefficients by calculating a piecewise SEM

275   with maximum likelihood estimation, using the piecewise SEM package (Lefcheck, 2015).

276   This is a useful tool for simultaneously testing complex multivariate hypotheses, using a set of

277   linear mixed-effect models, as it allows the inclusion of random factors. For the experimental

278   dataset, we included study site as a random factor in the underlying mixed models. As our *a*

279   *priori* model for the experimental dataset was saturated – i.e. there was a direct uni- or bi-

280   directional relationship between all variables, because all were plausible hypotheses – it was

281   not possible to perform the traditional goodness-of-fit test for the model.

282

283   **3. Results**

284   *3.1 Land-use effects on plant biomass, bryophyte species richness and bryophyte cover*

285   Land-use intensification strongly reduced bryophyte richness and cover. In the observational

286   dataset, low LUI plots (LUI <1.5; $N = 60$) harbored on average 3.9 (± 0.5 SE) bryophyte

287   species, while high LUI plots (LUI >1.5plots; $N = 84$) harbored 1.8 (± 1.4) species. Bryophyte

288   cover was on average 13.7% (± 2.3 SE) in low LUI plots, while in high LUI plots it was only

289   2.1% (± 0.1).

290   In the experimental dataset, mean bryophyte species richness ranged from an average of 9.8

291   (± 1.1) in the control plots to 3.1 (± 0.4) in the high intensity plots. Bryophyte cover ranged

292   from an average of 22.1% (± 7.3) and 26.0% (± 7.3) in the control and irrigation plots,

293   respectively, to 4.6% (± 1.9) in the high intensity plots.

294   In our linear models, plant biomass generally increased while bryophyte species richness and

295   cover decreased with increasing mowing-fertilization intensity (observational dataset; Tab. 2;

296   Supplementary material Fig. A.2; see also Tab. A.1 and Fig. A.3 for effects of increasing

297   LUI), and with higher amounts of added fertilizer (experimental dataset; Tab. 2;

298   Supplementary material Fig. A.4). Moreover, increasing grazing intensity also reduced

299   bryophyte species richness (observational dataset; Tab. 2). In the experimental dataset, we

300   found no effects of altitude and irrigation on plant biomass and bryophyte species richness

301   (Tab. 2; Fig. A.4).

302

303   *3.2 Direct versus indirect land-use effects on bryophyte species richness*

304   Land-use intensity had larger direct than indirect effects on bryophytes. In the observational

305   dataset, the structural equation modelling shows that the cumulative direct effect of

306   fertilization and mowing intensity (land-use intensity composite variable) on bryophyte

307   species richness was strongly negative and even stronger than the indirect effect mediated by

308   increased plant biomass (standardized effects: -0.37 vs. -0.05; Fig. 1A). In addition, we found

309   a moderate, negative effect of grazing intensity on bryophyte species richness.

310   In the experimental dataset, fertilization also directly decreased bryophyte species richness,

311   and this direct effect was again stronger than the indirect effect mediated by increased plant

312   biomass (-0.54 vs. -0.16; Fig. 1B). Irrigation, in contrast, had no direct or indirect effect on

313   bryophyte species richness.

314

315   **4. Discussion**

316   To increase productivity, grasslands are commonly fertilized and in dry areas, such as the

317   inner-alpine valleys of the Valais (SW Switzerland), they are often irrigated. As a result, these

318   grasslands can be mown more frequently or grazed with higher livestock densities. These

319   aspects of land-use intensification all seem to reduce grassland bryophyte species richness.

320   The one exception is irrigation, which had no effect on bryophyte species richness in the

321   Swiss Alps, which supports results from Müller et al. (2016) who found no effects of

322   irrigation on plant species richness in German lowland grasslands. Moreover, in our study

323   irrigation had no significant effect on bryophyte cover. This is in contrast to Virtanen et al.

324   (2017) who found an increasing bryophyte cover in only irrigated experimental plots under

325   Mediterranean climate conditions in California. However, high rates of fertilization seem to

326   be particularly negative for bryophytes.

327   In line with a number of other studies, we observed an overall decrease in bryophyte species

328   richness and cover with increasing fertilization. In particular, only very few species remained,

329   and at low abundance in strongly fertilized plots, such as *Eurhynchium* species or large-

330   growing *Brachythecium* species, which are known to tolerate fertilization (Dirkse and

331   Martakis, 1992; Nebel and Philippi, 2001). Other less competitive species suffered from even

332   very low amounts of fertilizer and were absent in our fertilized plots (e.g. *Aphanorrhegma*

333   *patens, Campylium calcareum*, *Ditrichum cylindricum, Leptobryum pyriforme, Phascum* spp.,

334   *Pottia* spp., *Pterygoneurum ovatum*). In addition, all 15 species listed as endangered or near

335   threatened in Germany (see Ludwig et al., 1996: *Campylium calcareum, C. chrysophyllum,*

336   *Ctenidium molluscum, Didymodon acutus, Rhytidium rugosum, Entodon concinnus, Fissidens*

337   *dubius, Homalothecium lutescens, Hylocomium splendens, Rhytidiadelphus triquetrus,*

338   *Thuidium abietinum, T. philibertii, Tortella tortuosa, Weissia brachycarpa, W. longifolia var.*

339   *longifolia*) occurred only in unfertilized plots, with a LUI below 1.5. *Homalothecium*

340   *lutescens* was present at low abundance in two plots and *Tortella tortuosa* in one plot with

341   low fertilizer input. Our results agree with several other observational and experimental

342   studies: Virtanen et al. (2000), working in the long-term Park Grass experiment in England,

343   and Bergamini and Pauli (2001), working in Swiss calcareous fens, both found a decline in

344   bryophyte richness with increasing fertilizer application. Müller et al. (2012) also observed a

345   decrease in bryophyte richness with increasing productivity, along a land-use intensity

346   gradient in German grasslands. Virtanen et al. (2017) found a reduction of bryophyte species

347 richness and cover in experimental plots, which were irrigated and fertilized. However, these

348 previous studies were not able to identify the mechanisms behind the impacts of fertilization.

349 By using SEM, we could separate direct fertilization, irrigation and grazing effects from the

350 indirect effects that are mediated by an increase in plant biomass, to better understand the

351 mechanisms underlying the effect of fertilization on bryophytes. Interestingly, we found that

352 the direct negative effect of fertilization and mowing in the observational dataset and

353 fertilization in the experimental dataset, was even stronger than the indirect negative effect

354 caused by increased plant biomass. The direct effects of fertilization could be explained by the

355 toxic effects of nitrogen: during the mineralization process, organic material or fertilizer is

356 first transformed into ammonia (ammonification) by saprobiotic bacteria, then to nitrite and

357 finally to nitrate by nitrifying bacteria (nitrification). While the enrichment of terrestrial

358 systems with nitrate mainly increases productivity (Humbert et al., 2016), the addition of

359 ammonia can have direct toxic effects on plants (e.g., disturbance of the ionic balance in

360 leaves, decreasing their longevity and growth: Roelofs et al., 1985). Bryophytes lack true

361 roots and vascular systems and therefore take up water and nutrients across their whole

362 surface, which may make bryophytes more sensitive to toxic fertilizer effects. Such toxic

363 fertilizer effects, in particular from ammonia, have been identified in experiments on selected

364 moss species (Krupa, 2003; Pearce et al., 2003; Paulissen et al., 2004, Andersen et al., 2016).

365 For example, Krupa (2003) reviewed effects of atmospheric ammonia on plants and reported

366 foliar damage on four moss species. Paulissen et al. (2004) and Andersen et al. (2016)

367 reported direct negative effects of ammonia on fen bryophyte species investigated in

368 greenhouse experiments. In addition, Verhoeven et al. (2011) found negative effects of

369 ammonia on bryophyte species richness in an experimental study from a fen in Ireland and

370 suggested a combination of increased competition with vascular plants and direct toxic

371 fertilizer effects as the two main causes.

In addition to these direct effects, fertilization did also indirectly reduce bryophyte species richness by increasing plant biomass. In all our study grasslands, plant biomass clearly increased with land-use intensification and in particular with larger fertilization inputs. This increase of plant growth and plant biomass following fertilization causes a loss of plant diversity, principally due to increased light competition and the shading out of understory plants by taller species (Hautier et al., 2009; Grace et al., 2016; DeMalach et al., 2017). The understory layer of grasslands, below the vascular plant canopy, is often formed of bryophytes. If we assume that plant biomass negatively correlates with the light available for bryophytes, our results suggest that an increase in light competition from vascular plants partially explains the negative effect of fertilization on bryophyte diversity. Feßel et al. (2016), who measured light transmittance to the ground in German grasslands, found sward cover and aboveground biomass – two positively related factors (Heer et al., 2018) – to be the most important factors explaining lower light levels on the ground, supporting our assumption that higher biomass means less light. Our results indicate that fertilization affects bryophyte diversity through several mechanisms and that the impacts may be more complex than previously thought.

The type of fertilizer may also be important and different fertilizers may vary in how much they reduce bryophyte diversity. In our plots, both in the experimental and in the observational study, liquid manure was the main type of added fertilizer, which consists of solid particles with high nutrient concentrations that cover the vegetation until the next rain event. It has been demonstrated that these solid components can directly kill bryophytes by osmotic effects, leading to so-called "browning" and this can strongly reduce their cover (Jäppinen and Hotanen, 1990). The underlying physiological mechanism of these toxic effects of fertilizer on bryophytes certainly needs more detailed, experimental investigations to be fully understood. However, our results already suggest that these effects are even more

397   important than the increased light competition by vascular plants in reducing bryophyte

398   diversity in intensively managed grasslands.

399   In the observational dataset, we found decreased bryophyte species richness at high land-use

400   intensity and that intensive grazing and mowing also reduced bryophyte diversity.

401   Disturbances caused by intensive mowing and grazing could therefore also contribute to the

402   decline of bryophyte species richness in intensively managed grassland: against the intuitive

403   expectation that higher mowing frequencies might be positive for bryophytes, because it

404   reduces plant cover and thereby increases the light levels at the ground, Müller et al. (2012)

405   found that bryophyte richness declined with increasing mowing frequency. However, as

406   frequently mown plots were also fertilized, it might well be that that this effect was driven by

407   the direct negative fertilizer effect. High grazing intensity can also reduce bryophyte richness

408   because of trampling and eutrophication (Pearce et al., 2010; Ludvíková et al., 2014),

409   however it is likely that low-intensity grazing would promote the highest bryophyte species

410   richness (Bergamini et al., 2001). This is probably due to the enhanced environmental

411   heterogeneity promoted by light grazing and because grazing animals increase light levels by

412   removing of vascular plant biomass (Borer et al., 2014). Other studies have also found

413   positive effects of grazing on bryophytes, for example Takala et al. (2014) found that cattle

414   grazing increased bryophyte species richness in Finnish semi-natural grasslands. We do not

415   have any grasslands which have been abandoned, so our grazing gradient is from lightly

416   grazed to intensively grazed and this probably explains the overall negative effect of grazing

417   on bryophyte richness. Factorial experiments have shown that grazing can also offset negative

418   effects of fertilization on vascular plant diversity to some extent (Borer et al., 2014) and it is

419   plausible that grazing could reduce negative effects of fertilization on bryophytes. However,

420   mowing frequency and grazing intensity are confounded with fertilization in our dataset –

421   because meadows are fertilized with the aim to increase yield and are therefore mown more

422 frequently or grazed more intensively than unfertilized ones – meaning that it is hard to

423 disentangle their effects and not possible to look for interactions between them. However,

424 given the strong negative, direct effects of fertilization on bryophytes it is not likely that

425 increased grazing would be able to completely offset the reduction in bryophyte diversity in

426 fertilized grasslands.

427

428 **5. Conclusions**

429 Our results challenge the widespread view that the negative effects of fertilization on

430 bryophyte diversity are mostly indirect and mediated by increased light competition with

431 vascular plants. In fact, direct effects, possibly mediated by fertilizer toxicity, can be equally,

432 if not more important. This means that biomass removal alone will not be enough to maintain

433 bryophyte diversity and that reducing fertilizer input is crucial. As bryophyte richness

434 strongly declined with land-use intensification, we recommend keeping fertilizer inputs as

435 low as possible, and reducing mowing frequency and grazing intensity in agricultural

436 grasslands to maintain bryophyte diversity.

437

446 Research (Agroscope les Fougères) for providing logistic support and Stéphane Mettaz for

447 maintaining the experiment in the Swiss grasslands.

448

449 **7. References**

450 Allan, E., Bossdorf, O., Dormann, C.F., Prati, D., Gossner, M.M., Tscharntke, T., Blüthgen,

451 N., Bellach, M., Birkhofer, K., Boch, S., Böhm, S., Börschig, C., Chatzinotas, A., Christ, S.,

452 Daniel, R., Diekötter, T., Fischer, C., Friedl, T., Glaser, K., Hallmann, C., Hodac, L., Hölzel,

453 N., Jung, K., Klein, A.M., Klaus, V.H., Kleinebecker, T., Krauss, J., Lange, M., Morris, E.K.,

454 Müller, J., Nacke, H., Pasalic, E., Rillig, M.C., Rothenwöhrer, C., Schall, P., Scherber, C.,

455 Schulze, W., Socher, S.A., Steckel, J., Steffan-Dewenter, I., Türke, M., Weiner, C.N., Werner,

456 M., Westphal, C., Wolters, V., Wubet, T., Gockel, S., Gorke, M., Hemp, A., Renner, S.C.,

457 Schöning, I., Pfeiffer, S., König-Ries, B., Buscot, F., Linsenmair, K.E., Schulze, E.D.,

458 Weisser, W.W., Fischer, M., 2014. Inter-annual variation in land-use intensity enhances

459 grassland multidiversity. Proc. Natl. Acad. Sci. U.S.A. 111, 308–313.

460 Andersen, D.K., Ejrnæs, R., Riis, T., 2016. N- and P-addition inhibits growth of rich fen

461 bryophytes. J. Bryol. 38, 127–137.

462 Andrey, A., Humbert, J.-Y., Arlettaz, R., 2016. Functional response of leaf- and planthoppers

463 to modern fertilisation and irrigation of hay meadows. Basic Appl. Ecol. 17, 627–637.

464 Aude, E., Ejrnæs, R., 2005. Bryophyte colonisation in experimental microcosms: the role of

465 nutrients, defoliation and vascular vegetation. Oikos 109, 323–330.

466 Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting Linear Mixed-Effects

467 Models Using lme4. J. Stat. Softw. 67, 1–48.

468 Bergamini, A., Peintinger, M., Schmid, B., Urmi, E., 2001. Effects of management and

469 altitude on bryophyte species diversity and composition in montane calcareous fens. Flora

470 196, 180–193.

471 Bergamini, A., Pauli, D., 2001. Effects of increased nutrient supply on bryophytes in montane

472 calcareous fens. J. Bryol. 23, 331–339.

473 Blüthgen, N., Dormann, C.F., Prati, D., Klaus, V.H., Kleinebecker, T., Hölzel, N., Alt, F.,

474 Boch, S., Gockel, S., Hemp, A., Müller, J., Nieschulze, J., Renner, S.C., Schöning, I.,

475 Schumacher, U., Socher, S.A., Wells, K., Birkhofer, K., Buscot, F., Oelmann, Y.,

476 Rothenwöhrer, C., Scherber, C., Tscharntke, T., Weiner, C.N., Fischer, M., Kalko, E.K.V.,

477 Linsenmair, K.E., Schulze, E.D., Weisser, W.W., 2012. A quantitative index of land-use

478 intensity in grasslands: integrating mowing, grazing and fertilization. Basic Appl. Ecol. 13,

479 207–220.

480 Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante,

481 M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.-W., Fenn, M., Gilliam,

482 F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition

483 effects on terrestrial plant diversity: a synthesis. Ecol. Appl. 20, 30–59.

484 Boch, S., Fischer, M., Knop, E., Allan, E., 2015. Endozoochory by slugs can increase

485 bryophyte establishment and species richness. Oikos 124, 331–336.

486 Boch, S., Prati, D., Schöning, I., Fischer, M., 2016. Lichen species richness is highest in non-

487 intensively used grasslands promoting suitable microhabitats and low vascular plant

488 competition. Biodivers. Conserv. 25, 225–238.

489 Boch, S., Allan, E., Birkhofer, K., Bossdorf, O., Blüthgen, N., Christ-Breulmann, S.,

490 Diekötter, T., Dormann, C.F., Gossner, M.M., Hallmann, C., Hemp, A., Hölzel, N., Jung, K.,

491 Klaus, V.H., Klein, A.M., Kleinebecker, T., Lange, M., Müller, J., Nacke, H., Prati, D.,

492   Renner, S.C., Rothenwöhrer, C., Schall, P., Schulze, E.-D., Socher, S.A., Tscharntke, T.,

493   Türke, M., Weiner, C.N., Weisser, W.W., Westphal, C., Wolters, V., Wubet, T., Fischer, M.,

494   2016b. Extensive und jährlich wechselnde Nutzungsintensität fördert den Artenreichtum im

495   Grünland. ANLiegen Nat. 38, 86–95.

496   Borer, E.T., Seabloom, E.W., Gruner, D.S., Harpole, W.S., Hillebrand, H., Lind, E.M., Adler,

497   P.B., Alberti, J., Anderson, M., Bakker, J.D., Biederman, L., Blumenthal, D., Brown, C.,

498   Brudvig, L.A., Buckley, Y.M., Cadotte, M., Chenglin, C., Cleland, E.E., Crawley, M.J.,

499   Daleo, P., Damschen, E.I., Davies, K.F., De Crappeo, N.M., Du, G., Firn, J., Hautier, Y.,

500   Heckman, R.W., Hector, A., Hille Ris Lambers, J., Iribarne, O., Klein, J.A., Knops, J.M.H.,

501   La Pierre, K.J., Leakey, A.D.B., Li, W., Mac Dougall, A.S., McCulley, R.L., Melbourne,

502   B.A., Mitchell, C.E., Moore, J.L., Mortensen, B., O'Halloran, L.R., Orrock, J.L., Pascual, J.,

503   Prober, S.M., Pyke, D.A., Risch, A.C., Schuetz, M., Smith, M.D., Stevensens, C.J., Sullivan,

504   L.L., Williams, R.J., Wragg, P. D., Wright, J.P., Yang, L.H., 2014. Herbivores and nutrients

505   control grassland plant diversity via light limitation. Nature 508, 517–520.

506   Carroll, J.A., Johnson, D., Morecroft, M., Taylor, A., Caporn, S.J.M., Lee, J.A., 2000. The

507   effect of long-term nitrogen additions on the bryophyte cover of upland acidic grasslands. J.

508   Bryol. 22, 83–89.

509   DeMalach, N., Zaady, E., Kadmon, R., 2017. Light asymmetry explains the effect of nutrient

510   enrichment on grassland diversity. Ecol. Lett. 20, 60–69.

511   DeMalach, N, Kadmon, R., 2017. Light competition explains diversity decline better than

512   niche dimensionality. Funct. Ecol. 31, 1834–1838.

513   Dengler, J., Löbel, S., Boch, S., 2006. Dry grassland communities of shallow, skeletal soils

514   (Sedo-Scleranthenea) in northern Europe. Tuexenia 26, 159–190.

515 Dengler, J., Biurrun, I., Apostolova, I., Baumann, E., Becker, T., Becker, U., Berastegi, A.,

516 Boch, S., Cancellieri, L., Dembicz, I., Didukh, Y.P., Dolnik, C., Ermakov, N., Filibeck, G.,

517 Garcia-Mijangos, I., Giusso del Galdo, G., Guarino, R., Janišová, M., Jaunatre, R., Jensen, K.,

518 Jeschke, M., Kącki, Z., Kozub, Ł., Kuzemko, A.A., Löbel, S., Pedashenko, H., Polyakova,

519 M., Ruprecht, E., Szabó, A., Vassilev, K., Velev, N., Weiser, F., 2016. Scale-dependent plant

520 diversity in Palaearctic grasslands: a comparative overview. Bull. Eurasian Dry Grassl. Group

521 31, 12–26.

522 Dirkse G.M., Martakis G.F.P., 1992. Effects of fertilizer on bryophytes in Swedish

523 experiments on forest fertilization. Biol. Cons. 59, 155–161.

524 Du, E., Liu, X., Fang, J., 2014. Effects of nitrogen additions on biomass, stoichiometry and

525 nutrient pools of moss *Rhytidium rugosum* in a boreal forest in Northeast China. Environ.

526 Pollut. 188, 166–171.

527 Feßel C., Meier I.C., Leuschner C., 2016. Relationship between species diversity, biomass

528 and light transmittance in temperate semi-natural grasslands: Is productivity enhanced by

529 complementary light capture? J. Veg. Sci. 27, 144–155.

530 Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G.,

531 Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells,K.,

532 Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W.,

533 2010.Implementing large-scale and long-term functional biodiversity research: thebiodiversity

534 exploratories. Basic Appl. Ecol. 11, 473–485.

535 Gossner, M.M., Lewinsohn, T., Kahl, T., Grassein, F., Boch, S., Prati, D., Birkhofer, K.,

536 Renner, S.C., Sikorski, J., Arndt, H., Baumgartner, V., Blaser, S., Blüthgen, N., Börschig, C.,

537 Buscot, F., Diekötter, T., Jorge, L.R., Jung, K., Keyel, A.C., Klein, A.-M., Klemmer, S.,

538 Krauss, J., Lange, M., Müller, J., Overmann, J., Pašalić, E., Penone, C., Perović, D., Purschke,

539   O., Schall, P., Socher, S.A., Sonnemann, I., Tschapka, M., Tscharntke, T., Türke, M., Venter,

540   P.C., Weiner, C.N., Werner, M., Wolters, V., Wurst, S., Westphal, C., Wubet, T., Fischer, M.,

541   Weisser, W.W., Allan, E., 2016. Land-use intensification causes homogenization of grassland

542   communities across trophic levels. Nature 540, 266–269.

543   Grace, J.B., 2006. Structural equation modeling and natural systems. Cambridge University

544   Press, Cambridge.

545   Grace, J. B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S.,

546   Hautier, Y., Hillebrand, H., Lind, E.M., Pärtel, M., Bakker, J.D., Buckley, Y.M., Crawley,

547   M.J., Damschen, E.I., Davies, K.F., Fay, P.A., Firn, J., Gruner, D.S., Hector, A., Knops, J.M.,

548   MacDougall, A.S., Melbourne, B.A., Morgan, J.W., Orrock, J.L., Prober, S.M., Smith, M.D.,

549   2016. Integrative modelling reveals mechanisms linking productivity and plant species

550   richness. Nature 529, 390–393.

551   Harpole, W.S., Tilman, D., 2007. Grassland species loss resulting from reduced niche

552   dimension. Nature 446, 791–793.

553   Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay,

554   P.A., Hautier, Y., Hillebrand, H., MacDougall, A.S., Seabloom, E.W., Bakker, J.D., Cadotte,

555   M.W., Chaneton, E.J., Chu, C., Hagenah, N., Kirkman, K., La Pierre, K.J., Moore, J.L.,

556   Morgan, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., 2017. Out of the

557   shadows: multiple nutrient limitations drive relationships among biomass, light and plant

558   diversity. Funct. Ecol. 31, 1839–1846.

559   Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity

560   loss after eutrophication. Science 324, 636–638.

561   Heer, N., Klimmek, F., Zwahlen, C., Fischer, M., Hölzel, N., Klaus, V.H., Kleinebecker, T.,

562   Prati, D., Boch, S., 2018. Hemiparasite-density effects on grassland plant diversity,

563   composition and biomass. Perspect. Plant Ecol. Evol. Syst. 32, 23–29.

564   Hejcman, M., Hejcmanová, P., Pavlů, V., Beneš, J., 2013. Origin and history of grasslands in

565   Central Europe - a review. Grass Forage Sci. 68, 345–363.

566   Humbert, J.-Y., Dwyer, J.M., Andrey, A., Arlettaz, R., 2016. Impacts of nitrogen addition on

567   plant biodiversity in mountain grasslands depend on dose, application duration and climate: a

568   systematic review. Glob. Chang. Biol. 22, 110–120.

569   Jäppinen, J.-P., Hotanen, J.-P., 1990. Effect of fertilization on the abundance of bryophytes in

570   two drained peatland forests in Eastern Finland. Ann. Bot. Fenn. 27, 93–108.

571   Kleijn, D., Kohler, F., Báldi, A., Batáry, P., Concepción, E.D., Clough, Y., Díaz, M., Gabriel,

572   D., Holzschuh, A., Knop, E., Kovács, A., Marshall, E.J.P., Tscharntke, T., Verhulst, J., 2008.

573   On the relationship between farmland biodiversity and land-use intensity in Europe. Proc. R.

574   Soc. Lond. 276, 903–909.

575   Koperski, M., Sauer, M., Braun, W., Gradstein, S.R., 2000. Referenzliste der Moose

576   Deutschlands. Schr.reihe Vegetationsk. 34, 1–519.

577   Krupa, S. V. 2003. Effects of atmospheric ammonia (NH3) on terrestrial vegetation: a review.

578   Environ. Pollut. 124, 179–221.

579   Lefcheck, J. S. 2015. piecewiseSEM: Piecewise structural equation modeling in R for

580   ecology, evolution, and systematics. Methods Ecol. Evol. 7, 573–579.

581   Lessard-Therrien, M., Humbert, J.-Y., Arlettaz, R., 2017. Experiment-based recommendations

582   for biodiversity-friendly management of mountain hay meadows. Appl. Veg. Sci. 20, 352–

583   362.

584 Ludvíková, V., Pavlů, V.V., Gaisler, J., Hejcman, M., Pavlů, L., 2014. Long term defoliation

585 by cattle grazing with and without trampling differently affects soil penetration resistance and

586 plant species composition in *Agrostis capillaris* grassland. Agric. Ecosyst. Environ. 197, 204–

587 211.

588 Ludwig, G., Düll, R., Philippi, G., Ahrens, M., Caspari, S., Koperski, M. Lütt, S., Schulz, F.,

589 Schwab, G., 1996. Rote Liste der Moose (*Anthocerophyta* et *Bryophyta*) Deutschlands. –

590 Schr.reihe Veg.kd. 28, 189–306.

591 Mamolos, A.P., Vasilikos, C.V., Veresoglou, D.S., 2005. Vegetation in contrasting soil water

592 sites of upland herbaceous grasslands and N: P ratios as indicators of nutrient limitation. Plant

593 Soil 270, 355–369.

594 Manning, P., Gossner, M.M., Bossdorf, O., Allan, E., Zhang, Y.-Y., Prati, D., Blüthgen, N.,

595 Boch, S., Böhm, S., Börschig, C., Hölzel, N., Jung, K., Klaus, V.H., Klein, A.M.,

596 Kleinebecker, T., Krauss, J., Lange, M., Müller, J., Pašalić, E., Socher, S.A., Tschapka, M.,

597 Türke, M., Weiner, C., Werner, M., Gockel, S., Hemp, A., Renner, S.C., Wells, K., Buscot,

598 F., Kalko, E.K.V., Linsenmair, K.E., Weisser, W.W., Fischer, M., 2015. Grassland

599 management intensification weakens the associations among the diversities of multiple plant

600 and animal taxa. Ecology 96, 1492–1501.

601 Melts, I., Lanno, K., Sammul, M., Uchida, K., Heinsoo, K., Kull, T., Laanisto, L., 2018.

602 Fertilising semi-natural grasslands may cause long-term negative effects on both biodiversity

603 and ecosystem stability. J. Appl. Ecol. DOI10.1111/1365-2664.13129.

604 Müller, I.B., Buhk, C., Lange, D., Entling, M.H., Schirmel, J., 2016. Contrasting effects of

605 irrigation and fertilization on plant diversity in hay meadows. Basic Appl. Ecol. 17, 576–585.

606 Müller, J., Klaus, V.H., Kleinebecker, T., Prati, D., Hölzel, N., Fischer, M., 2012. Impact of

607 land-use intensity and productivity on bryophyte diversity in agricultural grasslands. PLoS

608 ONE 7(12), e51520.

609 Nebel, M., Philippi, G., 2001. Die Moose Baden-Württembergs. Band 2: Spezieller Teil

610 (Bryophytina II, Schistostegales bis Hypnobryales). Ulmer, Stuttgart.

611 Oldén, A., Raatikainen, K.J., Tervonen, K., Halme, P., 2016. Grazing and soil pH are

612 biodiversity drivers of vascular plants and bryophytes in boreal wood-pastures. Agric.

613 Ecosyst. Environ. 222, 171–184.

614 Paulissen, M.P.C.P., Van Der Ven, P.J.M., Dees, A.J., Bobbink, R., 2004. Differential effects

615 of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen

616 input. New Phytol. 164, 451–458.

617 Pearce, I.S.K., Woodin, S.J., Van Der Wal, R., 2003. Physiological and growth responses of

618 the montane bryophyte *Racomitrium lanuginosum* to atmospheric nitrogen deposition. New

619 Phytol. 160, 145–155.

620 Pearce, I.S.K., Britton, A.J., Armitage, H.F.A., Jones, B., 2010. Additive impacts of nitrogen

621 deposition and grazing on mountain moss-sedge heath. Bot. Helv. 120, 129–137.

622 Pykälä, J., 2005. Plant species responses to cattle grazing in mesic semi-natural grassland

623 Agric. Ecosyst. Environ. 108, 109–117.

624 R Core Team 2016. R: a language and environment for statistical computing. – R Foundation

625 for Statistical Computing, Vienna.

626 Roelofs, J.G.M., Kempers, A.J., Houdijk, A.L.F.M., Jansen, J., 1985. The effect of air-borne

627 ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands. Plant Soil 84, 45–56.

628   Rosseel, Y., 2012. lavaan: An R Package for Structural Equation Modeling. J. Stat. Softw. 48,

629   1–36.

630   Shipley, B., 2002. Cause and correlation in biology: A user`s guide to path analysis, structural

631   equations and causal inference. Cambridge University Press, Cambridge.

632   Sinaj, S., Richner, W., Flisch, R., Charles, R., 2009. Données de base pour la fumure des

633   grandes cultures et des herbages (DBF-GCH). Rev. Suisse Agric. 41, 1–98

634   Socher, S.A., Prati, D., Boch, S., Müller, J., Klaus, V.H., Hölzel, N., Fischer, M. 2012. Direct

635   and productivity-mediated indirect effects of fertilization, mowing and grazing on grassland

636   species richness. J. Ecol. 100, 1391–1399.

637   Soliveres, S., Manning, P., Prati, D., Gossner, M.M., Alt, F., Arndt, H., Baumgartner, V.,

638   Binkenstein, J., Birkhofer, K., Blaser, S., Blüthgen, N., Boch, S., Böhm, S., Börschig, C.,

639   Buscot, F., Diekötter, T., Heinze, J., Hölzel, N., Jung, K., Klaus, V.H., Klein, A.-M.,

640   Kleinebecker, T., Klemmer, S., Krauss, J., Lange, M., Morris, E.K., Müller, J., Oelmann, Y.,

641   Overmann, J., Pašalić, E., Renner, S.C., Rillig, M.C., Schaefer, H.M., Schloter, M., Schmitt,

642   B., Schöning, I., Schrumpf, M., Sikorski, J., Socher, S.A., Solly, E.F., Sonnemann, I., Sorkau,

643   E., Steckel, J., Steffan-Dewenter, I., Stempfhuber, B., Tschapka, M., Türke, M., Venter, P.,

644   Weiner, C.N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, W., Wolters, V., Wubet, T.,

645   Wurst, S., Fischer, M., Allan, E., 2016a. Locally rare species influence grassland ecosystem

646   multifunctionality. Philos. Trans. R. Soc. B 371(1694), 20150269.

647   Soliveres, S., van der Plas, F., Manning, P., Prati, D., Gossner, M.M., Renner, S.C., Alt, F.,

648   Arndt, H., Baumgartner, V., Binkenstein, J., Birkhofer, K., Blaser, S., Blüthgen, N., Boch, S.,

649   Böhm, S., Börschig, C., Buscot, F., Diekötter, T., Heinze, J., Hölzel, N., Jung, K., Klaus,

650   V.H., Kleinebecker, T., Klemmer, S., Krauss, J., Lange, M., Morris, E.K., Müller, J.,

651   Oelmann, Y., Overmann, J., Pašalić, E., Rillig, M.C., Schaefer, H.M., Schloter, M., Schmitt,

B., Schöning, I., Schrumpf, M., Sikorski, J., Socher, S.A., Solly, E.F., Sonnemann, I., Sorkau, E., Steckel, J., Steffan-Dewenter, I., Stempfhuber, B., Tschapka, M., Türke, M., Venter, P., Weiner, C.N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, W., Wolters, V., Wubet, T., Wurst, S., Fischer, M., Allan, E., 2016b. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. Nature 536, 456–459.

Spitale, D., Petraglia, A., Tomaselli, M., 2009. Structural equation modelling detects unexpected differences between bryophyte and vascular plant richness along multiple environmental gradients. J. Biogeogr. 36, 745–755.

Sun, S.-Q., Wang, G.-X., Chang, S.X., Bhatti, J.S., Tian,W.-L., Luo, J., 2017. Warming and nitrogen addition effects on bryophytes are species- and plant community-specific on the eastern slope of the Tibetan Plateau. J. Veg. Sci. 28, 128–138.

Takala, T., Tahvanainen, T., Kouki, J., 2014. Grazing promotes bryophyte species richness in seminatural grasslands. Ann. Bot. Fenn. 51, 148–160.

Turetsky, M., 2003. The role of bryophytes in carbon and nitrogen cycling. Bryologist 106, 395–409.

van Klink, R., Boch, S., Buri, P., Rieder, N.S., Humbert, J.Y., Arlettaz, R., 2017. No detrimental effects of delayed mowing or uncut grass refuges on plant and bryophyte community structure and phytomass production in low-intensity hay meadows. Basic Appl. Ecol. 20, 1–9.

Verhoeven, J.T., Beltman, A.B., Dorland, E., Robat, S.A., Bobbink, R., 2011. Differential effects of ammonium and nitrate deposition on fen phanerogams and bryophytes. Appl. Veg. Sci. 14, 149–157.

Comparing the responses of bryophytes and short-statured vascular plants to climate shifts and

676   eutrophication

677   Virtanen, R., Eskelinen, A., Harrison, S., 2017. Comparing the responses of bryophytes and

678   short-statured vascular plants to climate shifts and eutrophication. Funct. Ecol. 31, 946–954.

679   Virtanen, R., Johnston, A.E., Crawley, M.J., Edwards, G.R., 2000. Bryophyte biomass and

680   species richness on the Park Grass Experiment, Rothamsted, UK. Plant Ecol. 151, 129–141.

681

682   **Table 1**: Main geographic and climatic characteristics of the study regions.

683   **Table 2**: Summary of linear mixed-effect models separating the effects of the combined

684   fertilization and mowing intensity (LUI Fert/Mow) and the grazing intensity on vascular plant

685   biomass, bryophyte species richness and bryophyte cover in our investigated meadows of the

686   observational dataset. Significant differences are indicated by bold p-values at $P < 0.05$. R^2

687   denotes the squared correlation coefficient between predicted and observed values.

688

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690 **Figure 1:** Structural equation model depicting direct and indirect effects of land-use

691 components on bryophyte species richness. Squares are observed variables. The hexagon is a

692 composite variable. Numbers adjacent to arrows show standardized path coefficients and the

693 width of the line is proportional to the size of the path coefficients. Black lines indicate

694 positive and grey lines negative relationships. Asterisks next to path coefficients indicate p-

695 values ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s. $P < 0.1$. The dashed arrows show co-

696 variances between factors. R^2 denotes the proportion of variance explained for the endogenous

697 variables. Standardized effects (direct times indirect effect) derived from the structural

698 equation models depicted above. **A)** Observational dataset showing the effects of the

699 composite variable land-use intensity – composed by fertilization (F) and mowing (M)

700 intensity – and grazing (G) intensity on plant biomass and bryophyte richness ($X^2 = 0.544$, $P =$

701 0.461, $df = 1$). **B)** Experimental dataset showing the effect of fertilization and irrigation on

702 plant biomass and bryophyte species richness.

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