Grazing intensity rather than host plant's palatability shape the community of arbuscular mycorrhizal fungi in a steppe grassland

Maede Faghihinia[1, 2], Yi Zou[1*], Yongfei Bai[3], Martin Dudáš[4], Rob Marrs[2], Philip L. Staddon[1,5,6]

[1] Department of Health and Environmental Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, Jiangsu, 215123, China;
[2] School of Environmental Sciences, University of Liverpool, Liverpool L69 3GP, UK;
[3] State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China;
[4] Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083 Praha 4 - Krč, Czech Republic;
[5] Countryside and Community Research Institute, University of Gloucestershire, Cheltenham, GL50 4AZ, UK;
[6] School for Agriculture, Food and the Environment, Royal Agricultural University, Cirencester, GL7 6JS, UK.
*For Corresponce. Email: yi.zou@xjtlu.edu.cn; Tel. +86 (0) 512 8188 0473.

Abstract

Arbuscular mycorrhizal fungi (AMF) are the predominant type of mycorrhizal fungi in roots and rhizosphere soil of grass species worldwide. Grasslands are currently experiencing increasing grazing pressure, but it is not yet clear how grazing intensity and host plant grazing preference by large herbivores interact with soil- and root-associated AMF communities. Here, we tested whether the diversity and community composition of AMF in the roots and rhizosphere soil of two dominant perennial grasses, grazed differently by livestock, change in response to grazing intensity. We conducted a study in a long-term field experiment in which seven levels of field-manipulated grazing intensities were maintained for 13 years in a typical steppe grassland in northern China. We extracted DNA from the roots and rhizosphere soil of two dominant grasses,

Leymus chinense (Trin.) Tzvel. and *Stipa grandis* P. Smirn, with contrasting grazing preference by sheep. AMF DNA from root and soil samples were then subjected to molecular analysis. Our results showed that AMF α-diversity (richness) at the virtual taxa (VT) level varied as a function of grazing intensity. Different VT showed completely different responses along the gradient, one increasing, one decreasing and others showing no response. Glomeraceae was the most abundant AMF family along the grazing gradient, which fits well with the theory of disturbance tolerance of this group. In addition, sheep grazing preference for host plants did not explain much of the variation in AMF α-diversity. However, the two grass species exhibited different AMF community composition in their roots and rhizosphere soils. Roots exhibited a lower α-diversity and higher β-diversity within the AMF community than soils. Overall, our results suggest that long-term grazing intensity might have changed the abundance of functionally-diverse AMF taxa in favor of those with disturbance-tolerant traits. We suggest our results would be useful in informing the choice of mycorrhizal fungi indicator variables when assessing the impacts of grassland management choices on grassland ecosystem functioning.

Keywords

Rhizosphere, α-diversity, β-diversity, fungal traits, Illumina sequencing, grazing preference

53 # Introduction

54

55 Arbuscular mycorrhizal fungi (AMF) are a key part of the soil-root biota, playing a crucial role in
56 maintaining grassland productivity and stability (Moora and Zobel, 2010; Asmelash et al., 2016).
57 AMF are the predominant type of mycorrhizal fungi in grasslands and form unique communities
58 in the roots and rhizosphere soil of grass species (Vályi et al., 2015; Dudinszky et al., 2019).
59 Grasslands are currently experiencing increasing grazing pressure, but it is not yet clear whether
60 and how grazing intensity by large herbivores drives AMF diversity and community composition.
61 The extent of the grazing impact on AMF function and community structure is primarily determined
62 by intensity of grazing (Ba et al., 2012; Faghihinia et al., 2020b; Yang et al., 2020) as it has
63 disparate effects on above- and below-ground biodiversity and productivity (Yan et al., 2013).
64 However, most studies compare the effects of grazing on AMF communities in grazed and un-
65 grazed plots (Murray et al., 2010; Guo et al., 2016; van der Heyde et al., 2017a), with very few
66 investigating impacts along a gradient of grazing pressure such as that ranging from light grazing
67 pressure through to overgrazing (Mendoza et al., 2011; Ba et al., 2012; Faghihinia et al., 2020a).
68 This is a major shortcoming as livestock grazing of grasslands is a major agriculture practice
69 worldwide (Conant, 2010; O'Mara, 2012). Besides, identification of AMF communities has mostly
70 been confined to spore isolation approaches, thus potentially losing much of the relative diversity
71 of the active component of the mycorrhizal communities that could be better captured through the
72 use of novel molecular techniques (Kusakabe et al., 2018; Dudinszky et al., 2019).

73

74 The effects of grazing on the AMF community can be highly species-specific. Grazing impacts will
75 depend on the host plant identity due to their different palatability/digestibility traits that influence
76 selection by grazing livestock (Vályi et al., 2015; González et al., 2018). Although AMF are

77 considered as non-specific symbiotic partners at species level (Smith and Read, 2008), non-
78 random patterns in host-fungal interaction suggests some degree of host specificity or preference
79 which is regulated by the functional characteristics of both partners (Vályi et al., 2015; Sepp et
80 al., 2019; Davison et al., 2020). For example, the use of pyrosequencing analysis of AMF
81 communities colonizing the roots of three common grass species (Poaceae) with different
82 sensitivities to trampling and grazing revealed that host plant identity is critical in shaping the AMF
83 community structure and composition (Vályi et al., 2015). It is also known that AMF root
84 colonization differs among certain grasses that are selected differentially by grazing livestock
85 (Cavagnaro et al., 2019). Cavagnaro et al. (2019) reported a highly significant decrease in AMF
86 colonization as a result of increased grazing intensity for species preferred by the grazers and
87 less pronounced effects for the less-preferred species. This evidence, therefore, suggests that
88 the effect of grazing on AMF community is also dependent on host plant identity. Given that
89 herbivory-caused defoliation limits below-ground carbon allocation from the plant to their
90 associated fungal partners (Yang et al., 2020), preferred or not preferred plant species by grazers
91 within a community could be expected to modify the rhizosphere differentially, thus creating
92 distinct associated AMF communities and mycorrhizal functioning.
93
94 There is also evidence that the AMF communities differ between the host roots and the
95 rhizosphere soil (Hempel et al., 2007; Li et al., 2018; Sepp et al., 2019). It has been suggested
96 that the AMF community structure in these two environments is driven by a number of different
97 factors, with the AMF community composition colonizing the roots mainly determined by the host
98 plant, while the AMF assemblage of the rhizosphere soil is more related to environmental
99 conditions including soil properties (Li et al., 2018; Stevens et al., 2020). However, most studies
100 have investigated AMF assemblages in either root (Vályi et al., 2015) or soil (van der Heyde et
101 al., 2017a), and few have assessed both simultaneously. Studies based on the identification of
102 root-colonizing and soil-borne AMF assemblages between plants with different grazing

103 preferences by livestock could provide further insights into the impact of grazing on AMF
104 communities and the underlying mechanisms. Changes in AMF community structure could be
105 used as an indicator of plant-soil system health.
106
107 The objective of this study, therefore, is to find out whether AMF communities in the roots and
108 rhizosphere soil of two dominant perennial grasses (*Leymus chinense* (Trin.) Tzvel. and *Stipa*
109 *grandis* P. Smirn) change as a function of grazing intensity. *L. chinense* is a highly mycorrhizal
110 and palatable bunchgrass while *S. grandis* has lower mycorrhizal colonization and low palatability
111 (Wang et al., 2014). We conducted a study in a long-term experimental site with a gradient of
112 seven grazing intensities maintained for 13 years at a typical steppe grassland in northern China.
113 In our study, grazing intensity is represented in a gradient of seven levels, which may be better
114 than traditional replicated designs that consider only extreme environmental conditions
115 (Cottingham et al., 2005; Kreyling et al., 2018), where the influence of intermediate levels of
116 grazing intensity may not be detected.
117
118 We extracted and sequenced AMF DNA from the root and rhizosphere soil samples of each
119 species and evaluated (1) how AMF communities associated with both root and rhizosphere soil
120 varied between *L. chinense* and *S. grandis* along the grazing gradient, and (2) how AMF α-
121 diversity and β-diversity were affected by grazing intensity and host plant grazing preferences by
122 sheep. We hypothesized that the sheep-preferred *L. chinense* would have a different composition
123 of AMF taxa in its root and rhizosphere soil than the less-preferred *S. grandis*. In addition, AMF
124 taxa respond differently to grazing intensity because of their different traits. To our knowledge,
125 this study is the first to thoroughly characterize the AMF community using molecular techniques
126 in two common grass species with different palatability and mycorrhizal dependence along a
127 grazing gradient in a typical temperate steppe. Such information will contribute to a deeper
128 understanding of changes within the AMF community caused by livestock grazing and

129 disturbance, provide opportunities for developing AMF ecological indicators, and improve
130 restoration strategies for reestablishment of native vegetation in temperate grasslands.
131

132 Materials and Methods

133 Study site

134

135 This study was set up in a steppe grassland in a semi-arid zone with continental climate located
136 at the Sino-German Inner Mongolia Grassland Ecosystem Research Station (IMGERS) in the
137 Xilin River Basin (116° 42′ E; 43° 38′ N), Inner Mongolia, China. We conducted our experiment in
138 seven plots, each with different levels of grazing intensities (GI); each plot contained a flat area
139 of 2 ha and was subjected to one level of grazing intensity, from 0 to 9 ewes ha^{-1} with interval
140 increases of 1.5 ewe ha^{-1} giving a range of grazing intensities: 0 (no grazing), 1.5 (very light), 3
141 (light), 4.5 (light-moderate), 6 (moderate), 7.5 (heavy) and 9 (overgrazing) (Appendix A-1, Fig S1).
142 The grassland was grazed by young female sheep (ewes) ca. 35 kg live-weight. The ewes were
143 put in plots for 90 days throughout the growing season (June-September) every year. The
144 different grazing intensity treatments have been run continuously for 13 years before our study in
145 2019, hence the impact of the differing grazing intensities will have stabilized (Li et al., 2017; Ren
146 et al., 2018). A detailed description of vegetation cover, climate, and soil characteristics of the
147 experimental site can be found in the supplementary information (Appendix A-1).

148

149 Root and soil sampling

150

151 Root and rhizosphere soil sampling of two dominant grass species, *S. grandis* and *L. chinense*,
152 was conducted in the middle of the growing season in July 2019. Three parallel transects, 50 m
153 apart, were set up in each experimental plot (100 m × 200 m). Transect lines were 150 m long
154 and nine sampling points were placed at regular distance along each transect. Along each
155 transect, nine *S. grandis* and nine *L. chinense* were collected with their roots and adhering soil
156 from 0 to 20 cm of depth with a garden trowel. Twenty-seven samples of root and adhering soil
157 from the same plant species were taken in each plot. Collected samples on each transect were
158 then mixed to give a composite soil and plant samples of the same species. A schematic
159 representation of the experimental design can be found in Appendix A-2, Fig S2.
160
161 Root samples were rinsed with tap water and non-lignified, light-fine secondary roots were hand-
162 picked from the main root and cut into 1 cm pieces. 10 g fresh weight of roots was sub-sampled
163 and stored at -80 °C for DNA extraction. Rhizosphere soil samples, i.e. soil tightly adhering to the
164 roots, were sampled, sieved (2-mm sieve) and frozen at -80 °C for DNA extraction and
165 sequencing.
166

167 Molecular analysis

168 DNA extraction and PCR

169 DNA extraction and PCR were conducted by Guangdong Magigene Biotechnology Co. Ltd.
170 (Guangzhou, China). Total genomic DNA was extracted from 84 samples (7 plots x 3 transects x
171 2 plant species x 2 environments (root vs. soil)), consisting of 42 rhizosphere soil samples and 42
172 root samples, using a DNA extraction kit (MinkaGene Bacterial DNA Kit). 6 of 42 rhizosphere soil
173 samples did not provide usable data and were removed from subsequent analysis (Appendix A-
174 3, Table S1). DNA amplification was then performed using AMF specific primers by nested
175 polymerase chain reaction (nested PCR). Nested PCR was carried out using BioRad S1000 (Bio-

176 Rad Laboratory, CA) and two sets of primers: AML1 (5'-ATCAACTTTCGATGGTAGGATAGA-
177 3')/AML2 (5'-GAACCCAAACACTTTGGTTTCC-3') (Lee et al., 2008) in first PCR and primers
178 AMV4.5NF (5′-AAGCTCGTAGTTGAATTTCG-3′) and AMDGR (5′-
179 CCCAACTATCCCTATTAATCAT-3′) (Sato et al., 2005) in the second PCR.

180

181 Premix Taq™ DNA polymerase (Takara Bio, USA) was used to provide nearly error-free
182 amplification and high amplicon yield. PCR reactions, containing 25 µl 2 x Premix Taq™
183 DNA polymerase, 1 µl each primer (10 mM) and 3 µl DNA (20 ng/µl) template in a volume of 50
184 µl, were amplified. For the nested PCRs, the DNA template was the first PCR product diluted 1:10
185 in ultrapure H2O. The thermocycler settings were as follows: 94°C for 3 min then 35 cycles at
186 94°C for 45 s, 51°C for 40 s, 72°C for 60 s, and a final elongation step by 72°C for 10 min for the
187 first PCR followed by 94°C for 3 min, then 35 cycles at 94°C for 40 s, 58°C for 60 s, and 72°C for
188 60 s and a final elongation step of 72°C for 10 min for the nested PCR.

189

190 The DNA fragments resulting from PCR were separated by size via agarose gel electrophoresis
191 within a 1.5% agarose gel in 0.5 × TAE (40 mM Tris-acetate, 1 mM EDTA) as running buffer. After
192 separation, the resulting DNA fragments were visible as clearly defined bands. The AxyPrepDNA
193 gel extraction kit was then used for the rapid purification of DNA fragments from TAE agarose
194 gels (AXYGEN, California, USA). The DNA concentrations of the purified PCR products were
195 quantified on the NanoDrop 1000™ Spectrophotometer (Thermo Fisher Scientific, MA, USA)
196 according to the manufacturer's instructions. PCR products was mixed in equimolar ratios
197 according to the GeneTools Analysis Software (Version4.03.05.0, SynGene). Then, mixture of
198 PCR products was purified with EZNA Gel Extraction Kit (Omega, USA). Sequencing libraries
199 were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England

200 Biolabs, USA) following manufacturer's recommendations and index codes were added. The
201 library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent
202 Bioanalyzer 2100 system.

203

204 ## Next-generation sequencing and species annotation

205

206 Illumina next-generation sequencing (NGS) technology was used for paired-end sequencing (2 ×
207 250 bp) on an Illumina NovaSeq™6000 sequencing platform. To check the quality of raw
208 sequence data and calculation of quality values, FastQC tool (Version 0.11.9) (Andrews, 2010)
209 was used. In addition, we used MultiQC (Ewels et al., 2016) to aggregate the reports from FastQC
210 into a single report with interactive plots for multiple bioinformatics analyses (Appendix B).

211

212 Following the quality check, CutAdapt (V 3.2) (Martin, 2011) was applied to cut adapters. Forward
213 and reverse reads were then processed using the SEED 2.1.1 pipeline (Větrovský and Baldrian,
214 2013); paired-end reads were merged and reads with an average quality below 30 and a length
215 of less than 200 bp, as well as sequences with ambiguous bases, were discarded. High quality
216 sequences were clustered into operational taxonomic units (OTUs) with 97% sequence identity
217 using the VSEARCH clustering program (Rognes et al., 2016). Representative sequences for
218 each OTU was screened for further annotation. We excluded singletons and doubletons as well
219 as OTUs that represented 0.002% of the total number of sequences to reduce the number of
220 spurious OTUs due to sequencing errors, following the recommendation of Bokulich et al. (2013).
221 Representative sequences of the OTUs were BLASTed (Altschul et al., 1990) against the
222 maarjAM database (Opik et al., 2010) to verify the Glomeromycotan origin of the sequences and

223 to categorize the reads to virtual taxa (VT). Raw sequencing data were deposited on the
224 Sequence Read Archive under accession number PRJNA758203.

225

226 Statistical analyses

227

228 We used three-way nested data to examine differences in AMF diversity and community
229 composition of the roots and rhizosphere soil from two different plant hosts. We also tested how
230 AMF α- and β-diversity at the virtual taxa (VT) level are affected by grazing intensity, host plant
231 identity, and mycorrhizal environment as well as their interactions. The data are nested in the
232 sense that samples were taken from seven sites (hereafter referred to as plots) with seven rates
233 of grazing intensity. Although replication is a key principle for dealing with random or spatially-
234 structured heterogeneity between individuals or experimental units, adding experimental units
235 along the gradient instead of increasing the number of replicates can improve the success of
236 overall prediction in ecological studies (Kreyling et al., 2018). In each plot, composite samples
237 were collected from two host plants (*L. chinense* and *S. grandis)* along three transects, and from
238 each host, samples included both roots and rhizosphere soil, where mycorrhizal community
239 composition was measured. See Appendix A-2 (Fig S2) for a schematic illustration of the
240 experimental design.

241

242 AMF α-diversity

243

244 Rarefaction curves were plotted to test how well a sample reflects the true diversity of root- and
245 soil- associated AMF and to compare observed OTUs richness among AMF communities in the
246 roots and rhizosphere soil (Fig 1).

247

248 AMF α-diversity was calculated using the richness and Shannon–Wiener diversity index based
249 on virtual taxa (VT). Linear mixed effects models were applied to test the impact of grazing
250 intensity on AMF Shannon diversity in the roots and rhizosphere soil of the two dominant plant
251 species. The response variable was the AMF diversity. Fixed explanatory variables were grazing
252 intensity, plant species, and mycorrhizal environment (root and rhizosphere soil) and the random
253 variable was transect ID (nested by grazing intensity). We first fitted a model with all terms as well
254 as all their interactions. Then, automated model selections using Akaike's Information Criterion
255 (AIC) (Burnham and Anderson, 2004) were conducted to select the best fitting model (Zuur et al.,
256 2009). Due to the design of this large-scale, long-term field experiment, grazing intensity was
257 treated as a continuous variable.

258

259 AMF β-diversity

260

261 To visualize the differences between AMF community composition in the root and rhizosphere
262 soil of the dominant plant species, non-metric multidimensional scaling (NMDS) was applied
263 based on the Bray-Curtis distance (Anderson et al., 2006).

264

265 To test how much of the variation in AMF community composition at VT level (β-diversity) was
266 explained by grazing intensity, mycorrhizal environment and host plant in the AMF community
267 data of all 78 samples (6 missing samples) at VT level, we performed redundancy analysis (RDA).
268 RDA was conducted on VT with relative abundance of >5% in soil and root. RDA was computed
269 based on Hellinger pre-transformed AMF composition data with three measured variables as
270 explanatory factors (Legendre and Gallagher, 2001). Monte Carlo permutation test with 999
271 permutations was applied to test the significance level for the variation explained by explanatory

272 variables (Zhu, 2005). We also calculated the significance of each constrained axis
273 independently. RDA ordinations were plotted as a triplot and type II scaling which was considered
274 as correlative relationships between variables.

275

276 All statistical analyses were carried out using R, version 3.6.3 (R Core Team, 2018). Generalized
277 linear mixed effect models were applied using *lme* function from “nlme” package (Pinheiro et al.,
278 2018). Automated model selection were carried out using *dredge()* function (Barton, 2018) from
279 “MuMIn” package. All models were validated by checking the distribution of residuals and
280 standard model validation graphs to verify homogeneity and normality and to identify influential
281 observations according to Zuur et al. (2009). Visual inspection of the residual plots did not show
282 any obvious deviations from homoscedasticity or normality. Species accumulation curves, NMDS,
283 RDA and the significance for constrained ordination test (Monte Carlo permutation test) were
284 conducted using functions available in “vegan” package (Oksanen, 2013).

285

286 Results

287 Characterization of AMF community

288

289 A total of 1,515,589 AMF sequences were obtained from 36 soil (6 samples failed to produce
290 useable data) and 42 root samples. 705376 and 810213 sequences appeared in soil and root
291 samples respectively. In addition, 727530 and 788059 sequences appeared in *S. grandis* and *L.*
292 *chinense* respectively. AMF sequences were then clustered into 1340 OTUs according to the ≥
293 97% similarity threshold. 1340 OTUs belong to 71 virtual taxa annotated in MaarjAM
294 Glomeromycota database. These VT belong to seven families (Fig 1): 61 Glomeraceae, 4

295 Claroideoglomeraceae, 2 Archaeosporaceae, 1 Ambisporaceae, 1 Paraglomeraceae, 1
296 Diversisporaceae, 1 Gigasporaceae.

297

298 The most abundant VT in soil were *Glomus_VTX00063* (12.11%), *Glomus_VTX00222* (10.47%),
299 *Glomus_VTX00167* (9.93%), *Glomus_VTX00329* (9.90%), *Glomus_VTX00156* (7.33%),
300 *Glomus_VTX00387* (5.50%) and *Glomus_VTX00304* (4.97%) respectively. The most abundant
301 VTs in roots were *Glomus_VTX00387* (25.87%), *Glomus_VTX00390* (11.76%),
302 *Glomus_VTX00156* (11.27%), *Glomus_VTX00167* (9.69%), *Glomus_VTX00304* (6.77%),
303 *Glomus_VTX00166* (5.83%) and *Glomus_VTX00386* (5.43%) respectively (Fig 1).

304

305 AMF α-diversity

306

307 Rarefaction analysis showed that the number of samples was sufficient to identify the major AMF
308 in the root and soil environment (Fig 2). The rarefaction curves of the AMF communities also
309 showed different OTU richness in the soil and root AMF communities; the AMF community had
310 greater species richness in the soil compared to the roots (Fig 2). The linear mixed effects models
311 revealed the significant effect of mycorrhizal environment on VT richness and VT diversity in the
312 best fitted models (Table 1). The significant effect of grazing intensity, however, was only
313 observed on VT richness. In addition, VT richness significantly impacted by host plant species,
314 with *L. chinense* harboring more VT than *S. grandis* (Appendix A-7, Fig S6).

315

316 AMF β-diversity

317

318 The NMDS plot of the similarity between AMF communities in the roots and rhizosphere soil of
319 the two dominant plant species showed a clear distinction between the mycorrhizal environment,
320 soil or root (shown with different colors), but the differences in grazing intensity (Appendix A-6,
321 Fig S5) and mycorrhizal communities between the two grass species were more subtle (visualized
322 as different shapes) as two groups of samples are mixed in the plot (Fig 3) (Appendix A-5, Fig
323 S4).

324

325 RDA analysis computed the proportion of variance in AMF composition at the VT level explained
326 by grazing intensity, mycorrhizal environment, and host plant. The analysis yielded three
327 canonical axes (RDA1 to RDA3) and three additional unconstrained axes for the residuals. The
328 three explanatory variables together explained 42% of the variance (P=0.001, adjusted R^2 =
329 0.394). The first (RDA1), second (RDA2), and third constrained axis (RDA3) explained 36.87%,
330 4.3%, and 0.59% of the variance, respectively. Calculating the significance of each constrained
331 axis using Monte Carlo permutation tests showed that the first (P=0.001) and second RDA
332 (P=0.001) axes were significant, but the third RDA was not (P=0.591). Furthermore, the RDA plot
333 clearly visualized the grazing intensity impact on the different AMF VT, e.g., the relative
334 abundance of *Glomus_VTX00390* was positively correlated with grazing intensity and the relative
335 abundance of *Glomus_VTX00329* was negatively correlated with grazing intensity (Fig 4).

336

337 Discussion

338

339 We determined AMF diversity and community composition in the roots and rhizosphere soil of two
340 dominant grasses with contrasting palatability/digestibility traits that influence different sheep-
341 grazing preferences within a 13-year grazing experiment with seven levels of grazing intensity on

342 a steppe grassland in China. Overall, we found long term grazing significantly affected AMF α-
343 diversity (VT richness), but Shannon diversity at VT was not significantly affected by grazing
344 intensity. The β-diversity (the relative abundance of AMF taxa) differed as a function of grazing
345 intensity. Grazing preference did not determine the composition of AMF taxa in two perennial
346 grass species. However, mycorrhizal environment, i.e. where sampling occurred, played a
347 determining role in shaping the AMF community composition.
348
349 While we acknowledge that true replications of grazing intensity on each plot would have
350 improved the statistical rigor of the work and allowed for the detection of differences among
351 grazing treatments, conducting replications would not have been feasible in a large-scale study
352 of this type, where plots must be relatively large (in this case, 2 ha per plot). In particular, a
353 replicated design would not exploit response patterns along the grazing gradient of interest in our
354 study (Kreyling et al., 2018).
355

356 AMF α-diversity and grazing intensity

357
358 AMF α-diversity (Shannon diversity at the VT level) was not significantly affected by grazing
359 intensity in our study (Table 1), confirming Ambrosino et al. (2018), who found no effect of grazing-
360 induced defoliation on diversity of AMF spores in Argentine pastures, and van der Heyde et al.
361 (2017a), who reported no effects of grazing on AMF communities detected in soil in Canadian
362 grasslands. However, we found a significant effect of grazing intensity on AMF richness (Table
363 1). There have been conflicting results from studies reporting either a positive or negative
364 response of AMF richness and diversity to grazing intensity (Ba et al., 2012; Bai et al., 2013).
365 These conflicting results may be caused by multiple approaches used to quantify AMF
366 communities, ranging from spore isolation to molecular techniques (van der Heyde et al., 2019).

367 Furthermore, Kusakabe et al. (2018) found different responses of AMF richness and diversity to
368 grazing intensity at three different grazing sites in Mongolian grasslands. They found a negative
369 correlation between grazing intensity and AMF diversity in one site and attributed this to decrease
370 in shoot biomass of mycorrhizal plant species and increase in non- or weakly mycorrhizal plants
371 (Kusakabe et al., 2018). In contrast, a positive correlation with grazing intensity in another site
372 was associated with higher AMF host biomass at the grazed sites (Kusakabe et al., 2018). Overall,
373 the grazing effects on AMF species diversity and underlying mechanisms are still controversial
374 as they depend on grazing-induced changes in mycorrhizal environment which varies along
375 different ecosystems (Faghihinia et al., 2020c).
376

377 AMF β-diversity and grazing intensity

378

379 It has been argued that traits of AMF communities vary in response to environmental disturbances
380 such as grazing, particularly at the species level (Faggioli et al., 2019). Our results showed various
381 responses among AMF virtual taxa to grazing intensity. We found significantly positive responses
382 of some taxa such as *Glomus_VTX00329* and significantly negative responses of some other
383 species such as *Glomus_VTX00390* to grazing intensity. Some taxa such as *Glomus_VTX00304*
384 fungi showed no remarkable variation in response to grazing intensity. Differential responses
385 among virtual AMF taxa to grazing-caused disturbance can be partly explained by differences in
386 their life-history traits such as growth rate, sporulation and resource conservation strategies (van
387 der Heyde et al., 2017b; Dudinszky et al., 2019). These traits most likely generate a wide range
388 of mycorrhizal functional groups, from grazing-tolerant to non-tolerant.
389

390 We found that the most abundant AMF VT in the roots and soil environments belonged to the
391 Glomeraceae and most of them were positively correlated with grazing intensity (Fig 4). The

392 Glomeraceae has often been described as the most disturbance-tolerant AMF family (Chagnon
393 et al., 2013; van der Heyde et al., 2017b; Stover et al., 2018). Glomeraceae is dominant in a broad
394 range of ecosystems worldwide, including agricultural systems, owning to their tolerance to
395 environmental disturbances achieved by its ruderal life-history strategy through high carbon use
396 efficiency, fast producing of large spores, and good adaptation to various host plants (Chagnon
397 et al., 2013; Ambrosino et al., 2018; Stevens et al., 2020). There is some evidence that AMF
398 species within the Glomeraceae tolerate consistent low levels of carbon supply from the host
399 plants as a result of herbivory and above-ground tissue loss, hence they are tolerant to grazing
400 (Stover et al., 2018; Dudinszky et al., 2019). In addition, the high rate of sporulation (Oehl et al.,
401 2009) and hyphal turnover (Staddon et al., 2003), as well as reproduction from both hyphal
402 fragments and spores probably makes AMF species of Glomeraceae resistant to hyphal
403 disruption and mycelial loss caused by grazing. Likewise, *Glomus* species were detected in
404 greater abundance in a long-term, overgrazed steppe compared with naturally-restored and non-
405 grazed sites in typical steppes of Inner Mongolia (Wang et al., 2014). These findings suggest that
406 functional life-history strategies in the Glomeraceae may have enhanced the capability of the most
407 species in this family to withstand grazing disturbance.
408
409 Taken together, our results suggest that long-term grazing intensity may have caused the
410 dominance of grazing-adapted mycorrhizal fungi in our experimental site. Therefore, grazing
411 intensity will have favored members of the AMF community with grazing-tolerant traits.
412
413 ## AMF and host plant grazing preference
414
415 Host plant identity and traits have been identified as an important driver of AMF community
416 structure in some cases (Martínez-García et al., 2015; Vályi et al., 2015). In an effort to investigate

417 the effects of land-use intensity on the AMF community of three common grass species with
418 contrasting mowing, trampling, and grazing tolerance in pastures with either mown or grazed, or
419 both management, Vályi et al. (2015) found that host plant-specific traits explained a large
420 proportion of variation in the AMF community in response to the type and degree of disturbance
421 at land-use sites. However, contrary to our prediction, we found no meaningful differences in AMF
422 diversity and community composition between the plant species we studied (*L. chinense* and *S.*
423 *grandis*) with contrasting grazing preferences by sheep. This can be explained by the fact that the
424 two grass species are grazing tolerant and remained the dominant plant species in all grazing
425 treatments despite subtle changes in their relative abundance (Li et al., 2017). Similar to our
426 findings, AMF spore abundance and diversity were not significantly affected by grazing in three
427 native perennial grass species with different livestock grazing preferences in Argentine
428 rangelands (Ambrosino et al., 2018). Given that the grasses we studied had different grazing
429 preferences by sheep, the lack of effect of host plant on AMF community composition suggests
430 that there is no strong preference among AMF communities or functional groups to colonize the
431 root and rhizosphere soil of sheep-preferred and non-preferred grass species. In fact, the
432 response of AMF to host plant grazing preferences by sheep appears to be similar within AMF
433 communities. Therefore, the effects of grazing on AMF community composition were independent
434 of the grazing preference of the dominant native grasses in our study site.
435
436 ## AMF community structure in soil and root
437
438 Consistent with previous findings, we found distinct AMF community structures in both the root
439 and rhizosphere soil, regardless of host plant identity (Hempel et al., 2007; Chen et al., 2014; Li
440 et al., 2018; Stevens et al., 2020). Not surprisingly, we detected lower α-diversity within the AMF
441 community in root compared to soil, suggesting that the AMF root community is less diverse in

442 terms of the number of different OTUs. The disparities between root and soil media are to be
443 expected, as these two mycorrhizal accessible environments represent different parts of the AMF
444 communities in intra-radical and extra-radical structures. Lower α-diversity in roots suggest that
445 some AMF species were dormant and inactive in the root system at the time of sampling (Hempel
446 et al., 2007; Vályi et al., 2015). This finding might also reflect the different AMF carbon allocation
447 to internal and external structures. Indeed, AMF invest either in long-lived internal structures or in
448 high-cost short-lived external ones depending on resource accessibility and environmental
449 condition (Johnson et al., 2010). The internal structures in roots, such as internal hyphae,
450 arbuscules, and, vesicules are those which are involved in transferring nutrients to the plant,
451 whereas external hyphal structure are involved in foraging soil and nutrient acquisition (Smith and
452 Read, 2008). Given that AMF cannot invest considerably in both internal and external components
453 simultaneously (Hart and Reader, 2002), some level of differential allocation to structures in soil
454 and root under grazing stress is highly likely. Furthermore, a greater homogeneity in root samples
455 in terms of AMF community composition can be interpreted as an evidence that AMF species in
456 roots are not random selections of AMF species from the soil. Therefore, AMF taxa may have
457 evolved with properties to colonize different mycorrhizal plants based on their life-history traits
458 and symbiont selection by their plant partners.

459

460 In contrast to α-diversity, we found higher β-diversity in the root samples compared to those of
461 soil. Although AMF community composition within the two environments did not vary at family
462 level, the relative abundance of AMF VT in the rhizosphere soils and roots were different (Fig 1).
463 The relative abundance of some VT are greater in soil samples (e.g. *Glomus_VTX00063* and
464 *Glomus_VTX00222*), whereas the relative abundance of some others are particularly greater in
465 root samples (e.g. *Glomus_VTX00387, Glomus_VTX00390* and *Glomus_VTX00156*) (Fig 1).
466 There are also some common VT that occur in root and soil with the same proportion, e.g.
467 *Glomus_VTX00167* (9.69% in root 9.93% in soil). This finding is expected as AMF isolates at the

468 species level differ considerably in the rate and extent of colonization in soil and root (Hart and
469 Reader, 2002). A greater proportion of some VT in root compared to soil, e.g. *Glomus_VTX00387*
470 (25.87% in root and 5.50% in soil) or *Glomus_VTX00390* (11.76% in root and in 3.7% soil)
471 indicates a strong plant dependency for these species, and potentially a less mutualistic
472 relationship.
473
474 In conclusion, by integrating the effects of grazing intensity, host plant, and assessment of
475 mycorrhizal community composition in different components (root vs. soil), we observed various
476 functional traits among AMF taxa at VT level that could explain some of the grazing responses.
477 We observed a significant increase in the relative abundances of most of AMF taxa belonging to
478 the Glomeraceae, such as *Glomus_VTX00390*. Some AMF taxa in contrast, decreased with
479 increasing grazing intensity, such as *Glomus_VTX00390*. The differences among AMF taxa in
480 their response to grazing stress reflect their various resource requirements and abilities to acquire
481 resources which affect their impacts on communities and ecosystems.
482
483 Grass species with contrasting grazing preferences by sheep did not differ in the composition of
484 their associated AMF communities in our study, suggesting that AMF functional groups do not
485 respond differently to host plant grazing preference. The mycorrhizal environment in which the
486 AMF community was measured (soil or root), regardless of host plant identity, determined to a
487 large degree the composition of AMF communities with lower Shannon diversity, higher β-
488 diversity in the root compared to the soil. Overall, our findings suggest that a decade of grazing
489 by large herbivores has resulted in functional changes in AMF communities in response to
490 grazing. Gaining knowledge on functionally- diverse traits of AMF taxa may improve our
491 understanding of the role of AMF in rehabilitating and restoring degraded grasslands around the

492 world. This would be beneficial to both the sustainable use of these grasslands for livestock
493 production and other ecosystem services such as climate change mitigation.
494
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496
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505 **Compliance with Ethical Standards**

506 **Competing Interests** The authors declare no competing interests of any sort.
507

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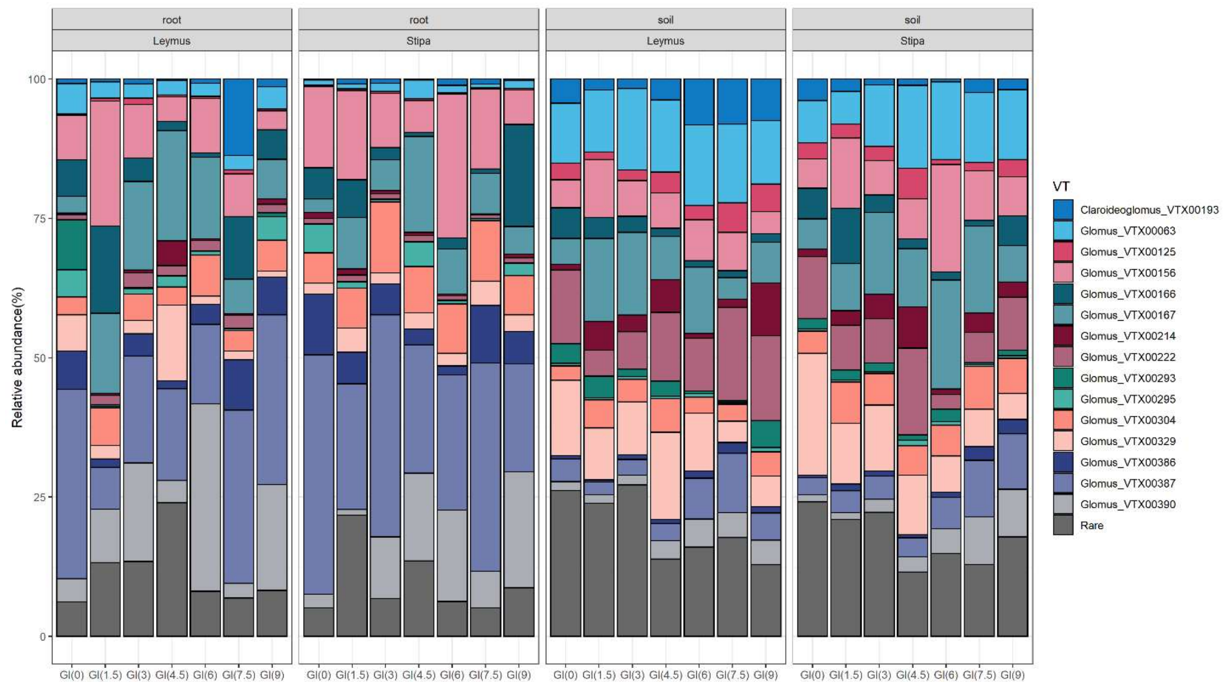
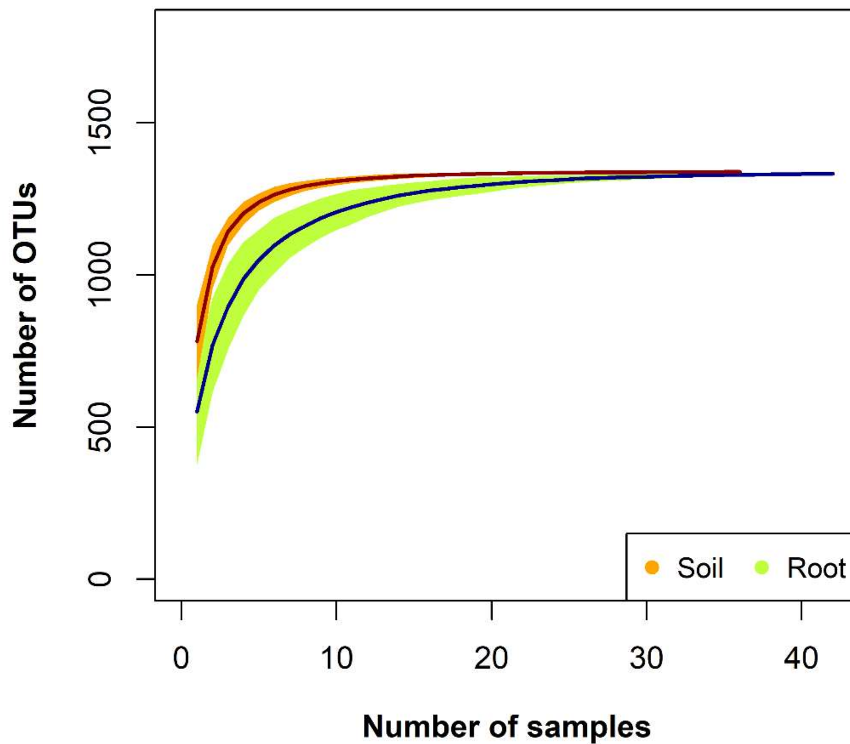


666 **Fig 1.** Proportion of reads of the most abundant virtual taxa (VT) (> 1 of relative
667 abundance) associated with the root and rhizosphere soil of two dominant grasses, S.
668 grandis and L. chinense, along the grazing gradient. VT with relative abundance less than
669 1 were grouped as rare species.

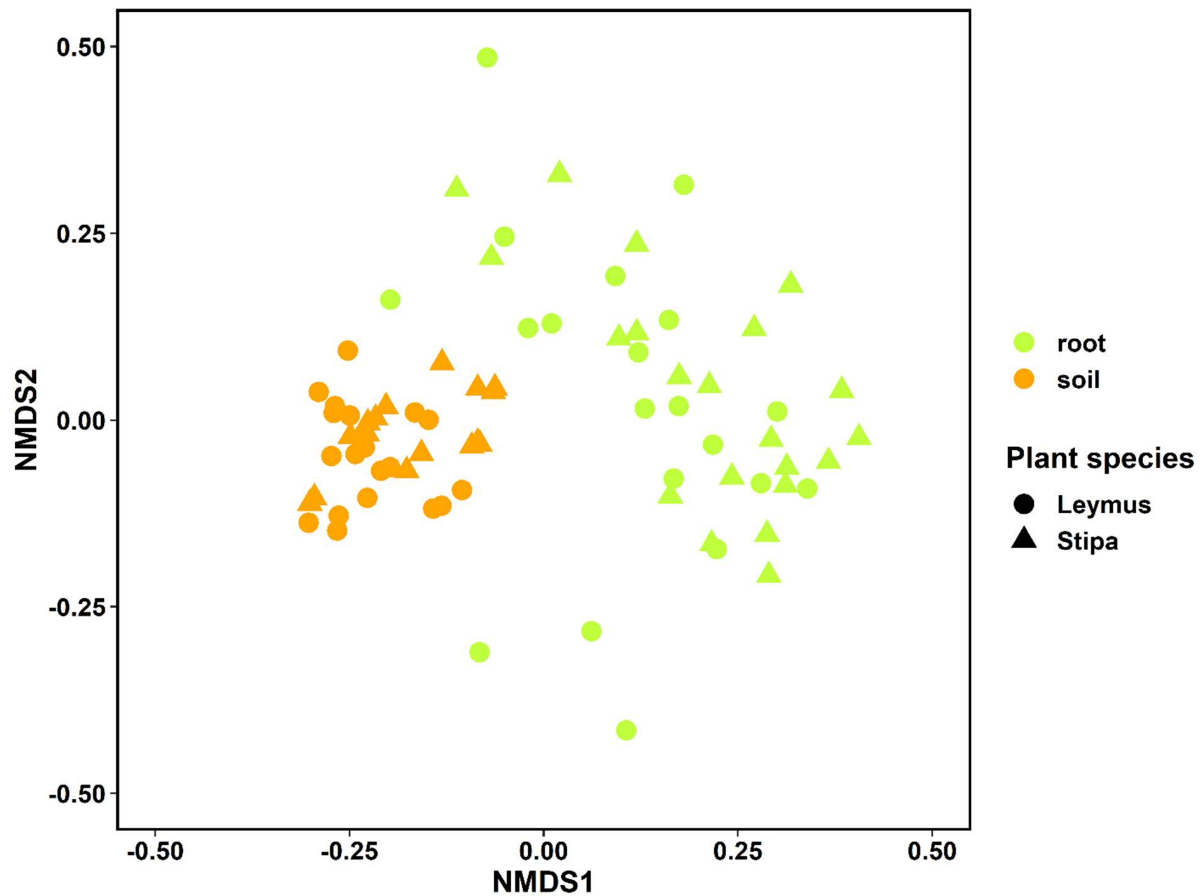
670



671

672 **Fig 2.** Species accumulation curves of estimated OTU richness for both root and
673 rhizosphere soil-associated AMF communities collected along a grazing gradient in a
674 steppe grassland in Inner Mongolia. Solid lines and light-colored region refer to the
675 average estimated richness and standard deviation, respectively.

676



677

678 **Fig 3.** Non-metric Multidimensional Scaling (NMDS, based on the Bray-Curtis dissimilarity
679 index) of root and rhizosphere soil-associated AMF communities collected along a
680 grazing gradient in a steppe grassland in Inner Mongolia (Stress values=0.167).

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Table 1. Linear mixed-effects model of the effects of grazing intensity, mycorrhizal environment and host plant identity on AMF richness and Shannon diversity. The full model (model No. 1) and the best model selected according Akaike's information criteria (AIC) (model No. 2) are presented; df is degrees of freedom; logLik is the log likelihood of the model; weight define a degree of belief (−△AIC/2) in each model where △AIC is the difference in AIC between a full model and the best model; Dashes (-) indicate variables that were not included in the model.

	Model No.	Grazing Intensity	Mycorrhizal Environment	Host plant	Grazing Intensity* Mycorrhizal Environment	Grazing Intensity* Host plant	Mycorrhizal Environment *Host plant	Grazing Intensity* Mycorrhizal Environment *Host plant	df	logLik	AICc	weight
VT richness	1	-17.67 ± 5.16 **(0.003)**	181.37 ± 39.12 **(0.000)**	-91.28 ± 38.09 **(0.020)**	8.55 ± 7.16 (0.238)	12.60 ± 7.04 (0.079)	136.73 ± 56.92 **(0.020)**	-14.18 ± 10.33 (0.176)	10	-438.70	900.7	0.036
	2	-10.66 ± 2.86 **(0.001)**	219.89 ± 21.91 **(0.000)**	-34.57 ± 21.33 (0.111)	-	-	72.73 ± 31.48 **(0.025)**	-	7	-440.50	896.6	0.276
VT diversity	1	-0.02 ± 0.02 (0.219)	0.79 ± 0.11 **(0.000)**	-0.21 ± 0.11 **(0.049)**	-0.00 ± 0.02 (0.823)	0.026 ± 0.02 (0.185)	0.14 ± 0.16 (0.372)	-0.02 ± 0.03 (0.577)	10	15.14	-7.0	0.005
	2	-	0.18± 0.02 **(0.000)**	-	-	-	-	-	4	11.34	-14.1	0.169

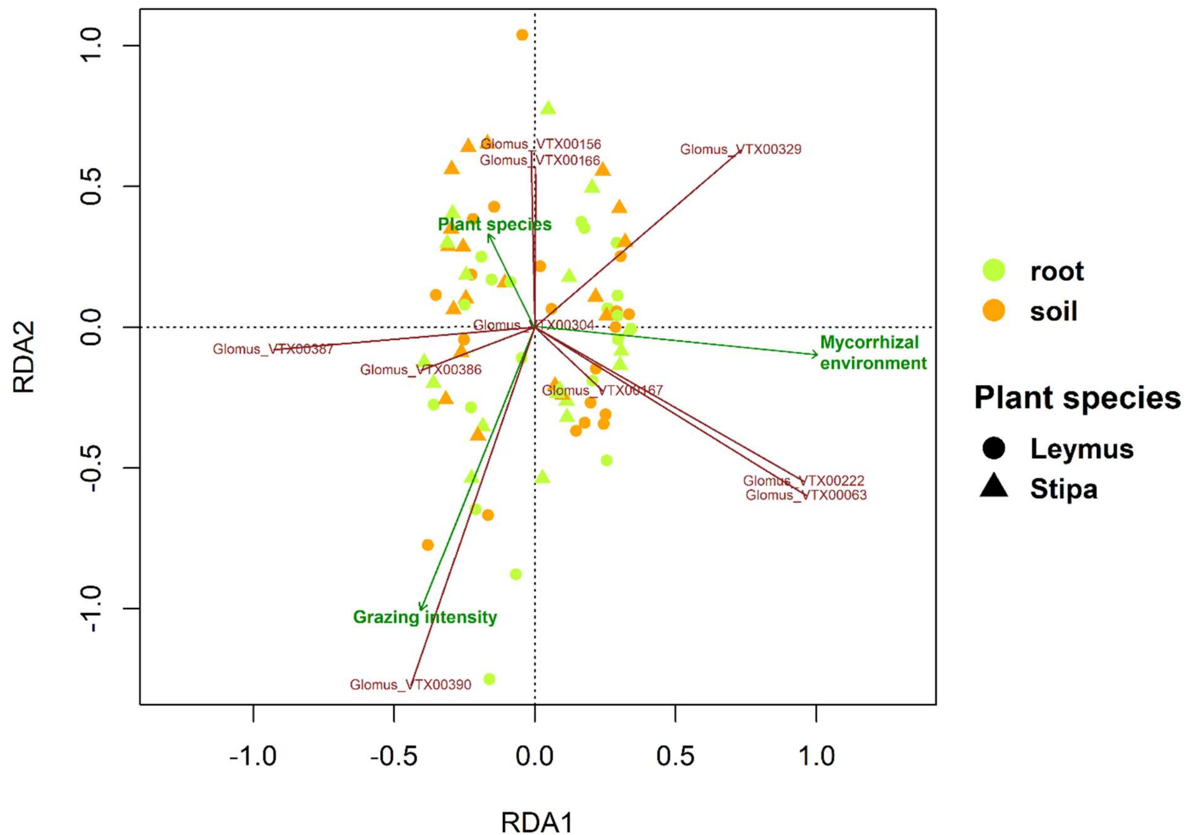

Fig 4. RDA triplot of 78 Illumina-sequenced root and soil samples collected along a grazing gradient in a steppe grassland in Inner Mongolia. Objects are ordinated as points while both response and explanatory variables are plotted as vectors. Explanatory environmental variables including grazing intensity, AMF environment and host identity are indicated by line arrows in green. The most abundant AMF VT (>5%) in root and soil environments are shown by brown arrows.

Supplementary data

Appendix A. Quality control of raw reads-MultiQC Report

FastQC tool was used to carry out the QC of the raw reads. We further used MultiQC (Ewels et al. 2016) to aggregate the reports from FastQC into a single report with interactive plots for multiple bioinformatics analyses.

Table 1- The general statistics table. %Dups**:** duplicated reads, %GC: average GC content, Length: average sequence length (bp), %Failed: percent of modules failed in FastQC report, M Seqs: total sequences (millions).

Sample Name	% Dups	% GC	Length	% Failed	M Seqs
F0_Lymus1_root_split_1_fq_gz	99.0%	48%	250 bp	36%	0.2
F0_Lymus1_root_split_2_fq_gz	96.7%	47%	250 bp	36%	0.2
F0_Lymus1_soil_split_1_fq_gz	97.9%	45%	250 bp	36%	0.2
F0_Lymus1_soil_split_2_fq_gz	93.9%	44%	250 bp	36%	0.2
F0_Lymus2_root_split_1_fq_gz	98.7%	45%	250 bp	36%	0.2
F0_Lymus2_root_split_2_fq_gz	95.5%	44%	250 bp	36%	0.2
F0_Lymus2_soil_split_1_fq_gz	98.1%	45%	250 bp	36%	0.2
F0_Lymus2_soil_split_2_fq_gz	94.3%	44%	250 bp	36%	0.2
F0_Lymus3_root_split_1_fq_gz	98.9%	46%	250 bp	36%	0.2
F0_Lymus3_root_split_2_fq_gz	95.9%	44%	250 bp	36%	0.2
F0_Lymus3_soil_split_1_fq_gz	98.2%	47%	250 bp	36%	0.1
F0_Lymus3_soil_split_2_fq_gz	94.8%	46%	250 bp	36%	0.1
F0_Stipa1_root_split_1_fq_gz	98.7%	44%	250 bp	36%	0.1
F0_Stipa1_root_split_2_fq_gz	95.5%	42%	250 bp	36%	0.1
F0_Stipa1_soil_split_1_fq_gz	98.1%	43%	250 bp	36%	0.1
F0_Stipa1_soil_split_2_fq_gz	93.5%	42%	250 bp	36%	0.1
F0_Stipa2_root_split_1_fq_gz	99.0%	47%	250 bp	36%	0.2
F0_Stipa2_root_split_2_fq_gz	96.2%	46%	250 bp	36%	0.2
F0_Stipa3_root_split_1_fq_gz	99.1%	46%	250 bp	36%	0.2
F0_Stipa3_root_split_2_fq_gz	96.7%	45%	250 bp	36%	0.2
F0_Stipa3_soil_split_1_fq_gz	98.1%	43%	250 bp	36%	0.1
F0_Stipa3_soil_split_2_fq_gz	93.6%	41%	250 bp	36%	0.1
F1_5_Lymus1_root_split_1_fq_gz	98.6%	42%	250 bp	36%	0.2
F1_5_Lymus1_root_split_2_fq_gz	95.4%	41%	250 bp	36%	0.2
F1_5_Lymus1_soil_split_1_fq_gz	98.3%	42%	250 bp	36%	0.1

Sample Name	% Dups	% GC	Length	% Failed	M Seqs
F1_5_Lymus1_soil_split_2_fq_gz	93.8%	41%	250 bp	36%	0.1
F1_5_Lymus2_root_split_1_fq_gz	98.7%	45%	250 bp	36%	0.2
F1_5_Lymus2_root_split_2_fq_gz	95.4%	43%	250 bp	36%	0.2
F1_5_Lymus3_root_split_1_fq_gz	98.8%	47%	250 bp	36%	0.2
F1_5_Lymus3_root_split_2_fq_gz	96.0%	46%	250 bp	36%	0.2
F1_5_Lymus3_soil_split_1_fq_gz	98.3%	44%	250 bp	36%	0.1
F1_5_Lymus3_soil_split_2_fq_gz	94.2%	42%	250 bp	36%	0.1
F1_5_Stipa1_root_split_1_fq_gz	98.5%	43%	250 bp	36%	0.2
F1_5_Stipa1_root_split_2_fq_gz	95.7%	41%	250 bp	36%	0.2
F1_5_Stipa2_root_split_1_fq_gz	98.8%	47%	250 bp	36%	0.1
F1_5_Stipa2_root_split_2_fq_gz	96.3%	47%	250 bp	36%	0.1
F1_5_Stipa2_soil_split_1_fq_gz	98.0%	43%	250 bp	36%	0.1
F1_5_Stipa2_soil_split_2_fq_gz	93.3%	42%	250 bp	36%	0.1
F1_5_Stipa3_root_split_1_fq_gz	98.7%	43%	250 bp	36%	0.1
F1_5_Stipa3_root_split_2_fq_gz	95.6%	42%	250 bp	36%	0.1
F1_5_Stipa3_soil_split_1_fq_gz	97.9%	43%	250 bp	36%	0.1
F1_5_Stipa3_soil_split_2_fq_gz	93.1%	42%	250 bp	36%	0.1
F3_Lymus1_root_split_1_fq_gz	98.7%	45%	250 bp	36%	0.2
F3_Lymus1_root_split_2_fq_gz	95.5%	44%	250 bp	36%	0.2
F3_Lymus1_soil_split_1_fq_gz	98.3%	44%	250 bp	36%	0.2
F3_Lymus1_soil_split_2_fq_gz	94.0%	43%	250 bp	36%	0.2
F3_Lymus2_root_split_1_fq_gz	98.6%	46%	250 bp	36%	0.1
F3_Lymus2_root_split_2_fq_gz	95.9%	45%	250 bp	36%	0.1
F3_Lymus2_soil_split_1_fq_gz	98.4%	43%	250 bp	36%	0.1
F3_Lymus2_soil_split_2_fq_gz	93.7%	42%	250 bp	36%	0.1
F3_Lymus3_root_split_1_fq_gz	98.5%	47%	250 bp	36%	0.1
F3_Lymus3_root_split_2_fq_gz	95.6%	46%	250 bp	36%	0.1
F3_Lymus3_soil_split_1_fq_gz	98.3%	44%	250 bp	36%	0.2
F3_Lymus3_soil_split_2_fq_gz	93.9%	42%	250 bp	36%	0.2
F3_Stipa1_root_split_1_fq_gz	98.8%	46%	250 bp	36%	0.2
F3_Stipa1_root_split_2_fq_gz	96.4%	46%	250 bp	36%	0.2
F3_Stipa1_soil_split_1_fq_gz	98.2%	42%	250 bp	36%	0.1
F3_Stipa1_soil_split_2_fq_gz	93.6%	40%	250 bp	36%	0.1
F3_Stipa2_root_split_1_fq_gz	98.8%	47%	250 bp	36%	0.1
F3_Stipa2_root_split_2_fq_gz	96.4%	47%	250 bp	36%	0.1
F3_Stipa2_soil_split_1_fq_gz	98.3%	43%	250 bp	36%	0.2

Sample Name	% Dups	% GC	Length	% Failed	M Seqs
F3_Stipa2_soil_split_2_fq_gz	94.0%	42%	250 bp	36%	0.2
F3_Stipa3_root_split_1_fq_gz	98.8%	47%	250 bp	36%	0.2
F3_Stipa3_root_split_2_fq_gz	96.1%	46%	250 bp	36%	0.2
F3_Stipa3_soil_split_1_fq_gz	98.3%	42%	250 bp	36%	0.1
F3_Stipa3_soil_split_2_fq_gz	93.8%	41%	250 bp	36%	0.1
F4_5Lymus1_soil_split_1_fq_gz	98.3%	42%	250 bp	36%	0.1
F4_5Lymus1_soil_split_2_fq_gz	93.6%	41%	250 bp	36%	0.1
F4_5Lymus2_soil_split_2_fq_gz	93.8%	40%	250 bp	36%	0.2
F4_5Lymus3_soil_split_1_fq_gz	98.5%	42%	250 bp	36%	0.2
F4_5Lymus3_soil_split_2_fq_gz	94.4%	41%	250 bp	36%	0.2
F4_5_Lymus1_root_split_1_fq_gz	98.8%	44%	250 bp	36%	0.2
F4_5_Lymus1_root_split_2_fq_gz	95.7%	42%	250 bp	36%	0.2
F4_5_Lymus2_root_split_1_fq_gz	98.5%	47%	250 bp	36%	0.1
F4_5_Lymus2_root_split_2_fq_gz	95.9%	46%	250 bp	36%	0.1
F4_5_Lymus3_root_split_1_fq_gz	98.8%	40%	250 bp	36%	0.1
F4_5_Lymus3_root_split_2_fq_gz	95.1%	39%	250 bp	36%	0.1
F4_5_Stipa1_root_split_1_fq_gz	98.8%	44%	250 bp	36%	0.1
F4_5_Stipa1_root_split_2_fq_gz	95.5%	43%	250 bp	36%	0.1
F4_5_Stipa1_soil_split_1_fq_gz	98.0%	43%	250 bp	36%	0.2
F4_5_Stipa1_soil_split_2_fq_gz	93.9%	41%	250 bp	36%	0.2
F4_5_Stipa2_root_split_1_fq_gz	98.8%	43%	250 bp	36%	0.2
F4_5_Stipa2_root_split_2_fq_gz	95.8%	42%	250 bp	36%	0.2
F4_5_Stipa2_soil_split_1_fq_gz	98.5%	41%	250 bp	36%	0.2
F4_5_Stipa2_soil_split_2_fq_gz	94.3%	41%	250 bp	36%	0.2
F4_5_Stipa3_root_split_1_fq_gz	98.8%	42%	250 bp	36%	0.1
F4_5_Stipa3_root_split_2_fq_gz	95.2%	42%	250 bp	36%	0.1
F4_5_Stipa3_soil_split_1_fq_gz	98.4%	42%	250 bp	36%	0.1
F4_5_Stipa3_soil_split_2_fq_gz	93.7%	41%	250 bp	36%	0.1
F6_Lymus1_root_split_1_fq_gz	98.8%	46%	250 bp	36%	0.2
F6_Lymus1_root_split_2_fq_gz	96.4%	45%	250 bp	36%	0.2
F6_Lymus2_root_split_1_fq_gz	98.9%	44%	250 bp	36%	0.2
F6_Lymus2_root_split_2_fq_gz	95.6%	43%	250 bp	36%	0.2
F6_Lymus2_soil_split_1_fq_gz	98.2%	43%	250 bp	36%	0.2
F6_Lymus2_soil_split_2_fq_gz	93.8%	42%	250 bp	36%	0.2
F6_Lymus3_root_split_1_fq_gz	98.8%	44%	250 bp	36%	0.1
F6_Lymus3_root_split_2_fq_gz	95.7%	43%	250 bp	36%	0.1

Sample Name	% Dups	% GC	Length	% Failed	M Seqs
F6_Lymus3_soil_split_1_fq_gz	98.6%	43%	250 bp	36%	0.2
F6_Lymus3_soil_split_2_fq_gz	94.4%	42%	250 bp	36%	0.2
F6_Stipa1_root_split_1_fq_gz	98.9%	43%	250 bp	36%	0.2
F6_Stipa1_root_split_2_fq_gz	96.2%	43%	250 bp	36%	0.2
F6_Stipa2_root_split_1_fq_gz	98.7%	44%	250 bp	36%	0.1
F6_Stipa2_root_split_2_fq_gz	95.3%	44%	250 bp	36%	0.1
F6_Stipa3_root_split_1_fq_gz	98.8%	46%	250 bp	36%	0.2
F6_Stipa3_root_split_2_fq_gz	96.2%	45%	250 bp	36%	0.2
F6_Stipa3_soil_split_1_fq_gz	98.3%	44%	250 bp	36%	0.1
F6_Stipa3_soil_split_2_fq_gz	94.2%	43%	250 bp	36%	0.1
F7_5_Lymus1_root_split_1_fq_gz	98.8%	43%	250 bp	36%	0.2
F7_5_Lymus1_root_split_2_fq_gz	96.3%	42%	250 bp	36%	0.2
F7_5_Lymus1_soil_split_1_fq_gz	98.0%	44%	250 bp	36%	0.1
F7_5_Lymus1_soil_split_2_fq_gz	94.0%	43%	250 bp	36%	0.1
F7_5_Lymus2_root_split_1_fq_gz	98.6%	44%	250 bp	36%	0.2
F7_5_Lymus2_root_split_2_fq_gz	96.2%	43%	250 bp	36%	0.2
F7_5_Lymus2_soil_split_1_fq_gz	98.1%	43%	250 bp	36%	0.2
F7_5_Lymus2_soil_split_2_fq_gz	94.0%	42%	250 bp	36%	0.2
F7_5_Lymus3_root_split_1_fq_gz	98.9%	41%	250 bp	36%	0.2
F7_5_Lymus3_root_split_2_fq_gz	96.8%	39%	250 bp	36%	0.2
F7_5_Lymus3_soil_split_1_fq_gz	97.8%	44%	250 bp	36%	0.1
F7_5_Lymus3_soil_split_2_fq_gz	93.9%	43%	250 bp	36%	0.1
F7_5_Stipa1_root_split_1_fq_gz	98.9%	43%	250 bp	36%	0.2
F7_5_Stipa1_root_split_2_fq_gz	96.1%	42%	250 bp	36%	0.2
F7_5_Stipa1_soil_split_1_fq_gz	97.9%	43%	250 bp	36%	0.1
F7_5_Stipa1_soil_split_2_fq_gz	93.4%	42%	250 bp	36%	0.1
F7_5_Stipa2_root_split_1_fq_gz	99.1%	40%	250 bp	36%	0.1
F7_5_Stipa2_root_split_2_fq_gz	96.5%	38%	250 bp	36%	0.1
F7_5_Stipa2_soil_split_1_fq_gz	98.2%	43%	250 bp	36%	0.2
F7_5_Stipa2_soil_split_2_fq_gz	93.8%	42%	250 bp	36%	0.2
F7_5_Stipa3_root_split_1_fq_gz	99.1%	39%	250 bp	36%	0.2
F7_5_Stipa3_root_split_2_fq_gz	96.3%	38%	250 bp	36%	0.2
F7_5_Stipa3_soil_split_1_fq_gz	98.0%	42%	250 bp	36%	0.2
F7_5_Stipa3_soil_split_2_fq_gz	94.0%	42%	250 bp	36%	0.2
F9_Lymus1_soil_split_1_fq_gz	98.3%	41%	250 bp	36%	0.2
F9_Lymus1_soil_split_2_fq_gz	94.0%	40%	250 bp	36%	0.2

Sample Name	% Dups	% GC	Length	% Failed	M Seqs
F9_Lymus2_soil_split_1_fq_gz	98.2%	42%	250 bp	36%	0.2
F9_Lymus2_soil_split_2_fq_gz	93.8%	41%	250 bp	36%	0.2
F9_Lymus3_soil_split_1_fq_gz	98.3%	43%	250 bp	36%	0.2
F9_Lymus3_soil_split_2_fq_gz	94.2%	42%	250 bp	36%	0.2
F9_Stipa1_root_split_1_fq_gz	98.7%	43%	250 bp	36%	0.1
F9_Stipa1_root_split_2_fq_gz	95.1%	42%	250 bp	36%	0.1
F9_Stipa1_soil_split_1_fq_gz	98.5%	41%	250 bp	36%	0.2
F9_Stipa1_soil_split_2_fq_gz	94.4%	40%	250 bp	36%	0.2
F9_Stipa2_root_split_1_fq_gz	98.8%	45%	250 bp	36%	0.1
F9_Stipa2_root_split_2_fq_gz	96.3%	44%	250 bp	36%	0.1
F9_Stipa2_soil_split_1_fq_gz	98.3%	42%	250 bp	36%	0.1
F9_Stipa2_soil_split_2_fq_gz	93.7%	41%	250 bp	36%	0.1
F9_Stipa3_root_split_1_fq_gz	98.6%	44%	250 bp	36%	0.2
F9_Stipa3_root_split_2_fq_gz	95.5%	43%	250 bp	36%	0.2
F9_Stipa3_soil_split_1_fq_gz	98.3%	42%	250 bp	36%	0.2
F9_Stipa3_soil_split_2_fq_gz	93.6%	41%	250 bp	36%	0.2
F_9_Lymus1_root_split_1_fq_gz	98.9%	44%	250 bp	36%	0.2
F_9_Lymus1_root_split_2_fq_gz	95.7%	43%	250 bp	36%	0.2
F_9_Lymus2_root_split_1_fq_gz	98.9%	45%	250 bp	36%	0.2
F_9_Lymus2_root_split_2_fq_gz	95.2%	44%	250 bp	36%	0.2
F_9_Lymus3_root_split_1_fq_gz	98.8%	45%	250 bp	36%	0.2
F_9_Lymus3_root_split_2_fq_gz	95.8%	44%	250 bp	36%	0.2

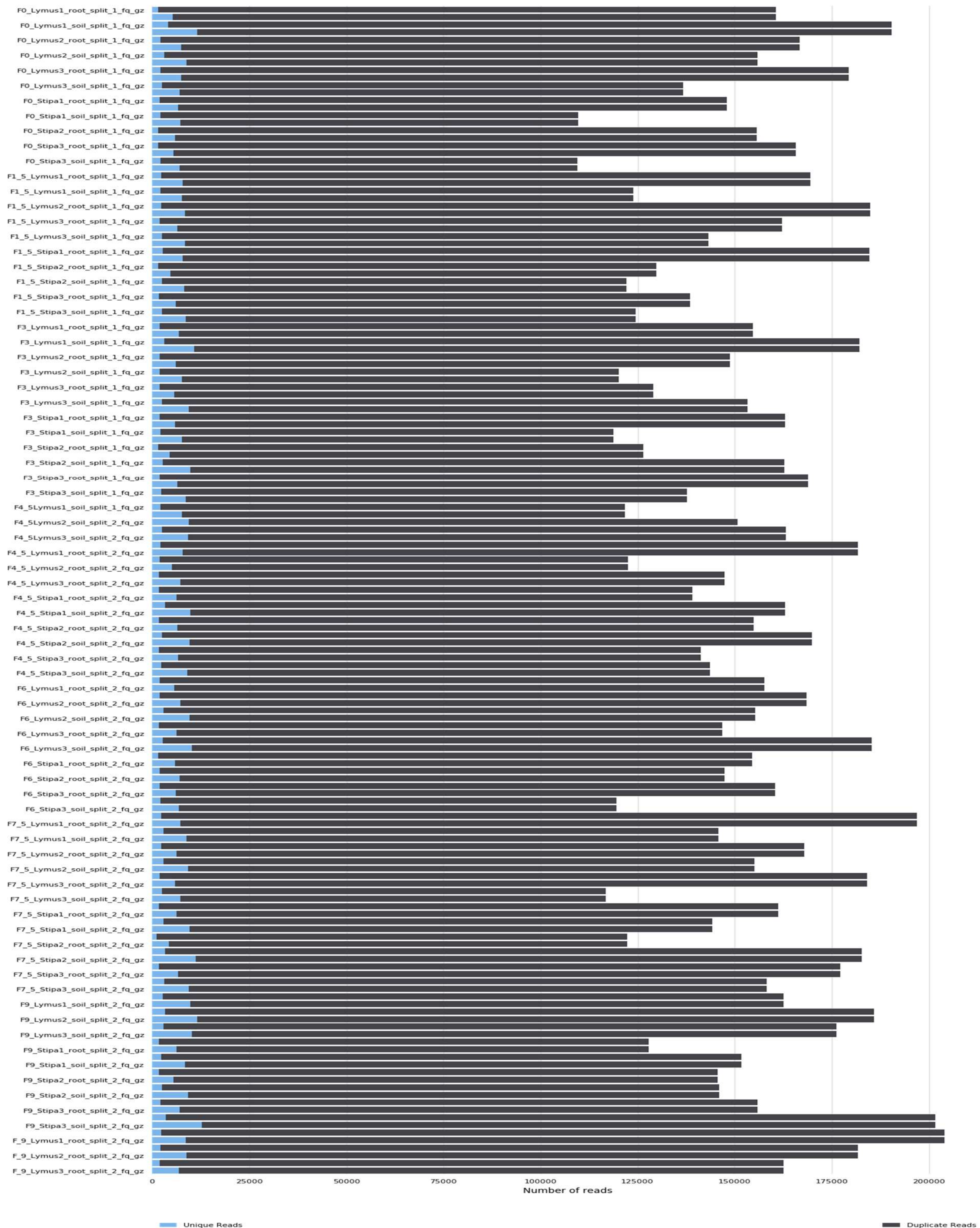

Figure 1. Sequence counts for each sample. Duplicate read counts are an estimate only. This plot shows the total number of reads, broken down into unique and duplicate reads. Only sequences which first appear in the first 100,000 sequences in each file were analysed. This should be enough to get a good impression for the

duplication levels in the whole file. Each sequence is tracked to the end of the file to give a representative count of the overall duplication level. The duplication detection requires an exact sequence match over the whole length of the sequence. Any reads over 75bp in length are truncated to 50bp for this analysis.

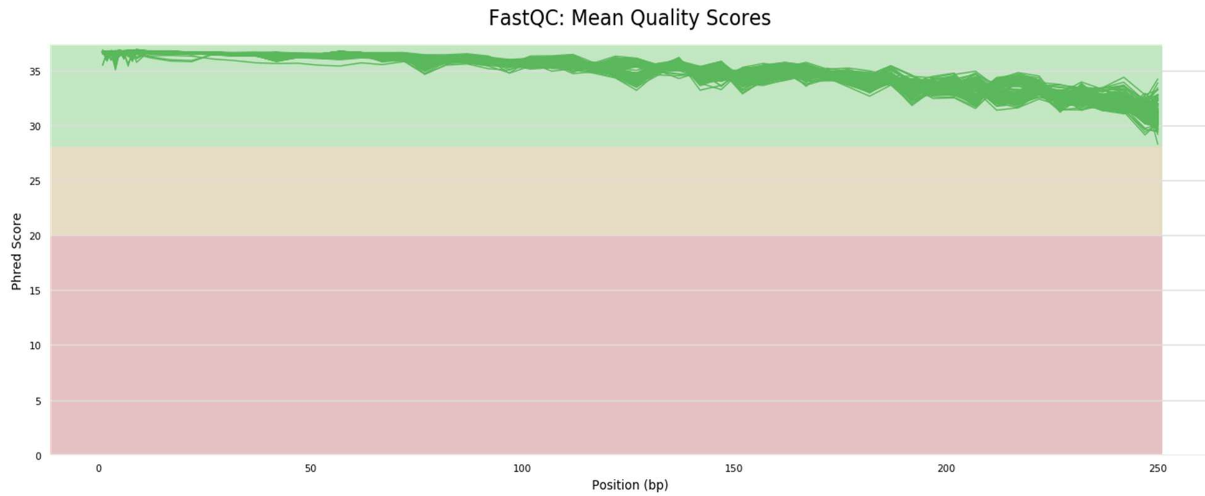

Figure 2. Sequence quality histograms. The mean quality value across each base position in the read. To enable multiple samples to be plotted on the same graph, only the mean quality scores are plotted. The y-axis on the graph shows the quality scores. The higher the score, the better the base call. The background of the graph divides the y axis into very good quality calls (green), calls of reasonable quality (orange), and calls of poor quality (red).

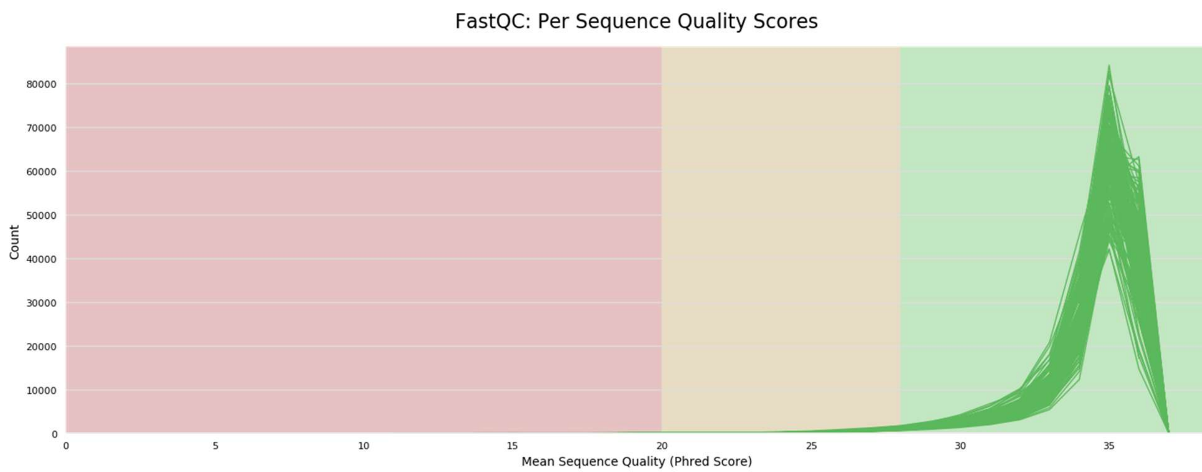

Figure 3. Per sequence quality scores. The graph shows the number of reads with average quality scores and a subset of reads with poor quality.

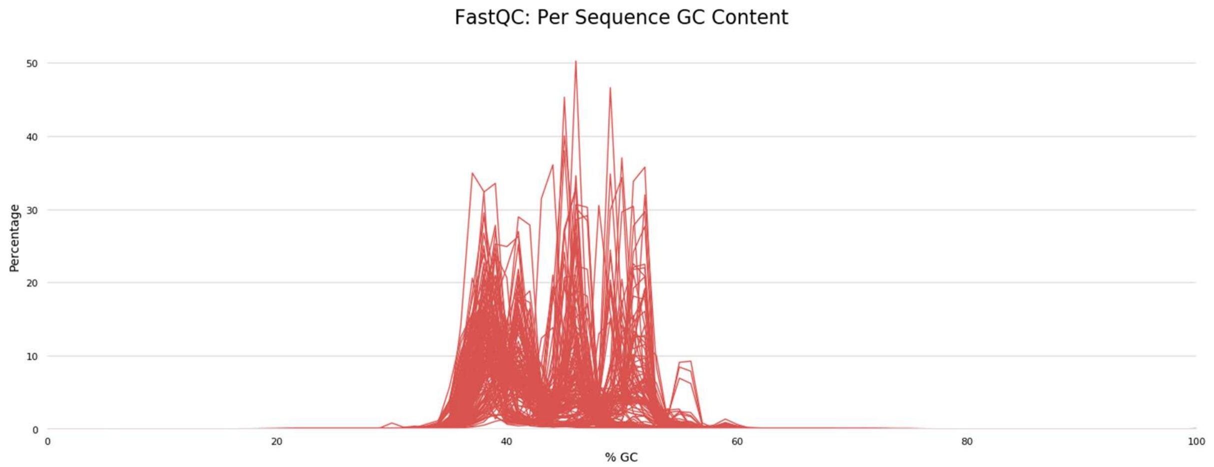

Figure 4. Per Sequence GC Content. The graph shows the average GC content of reads.

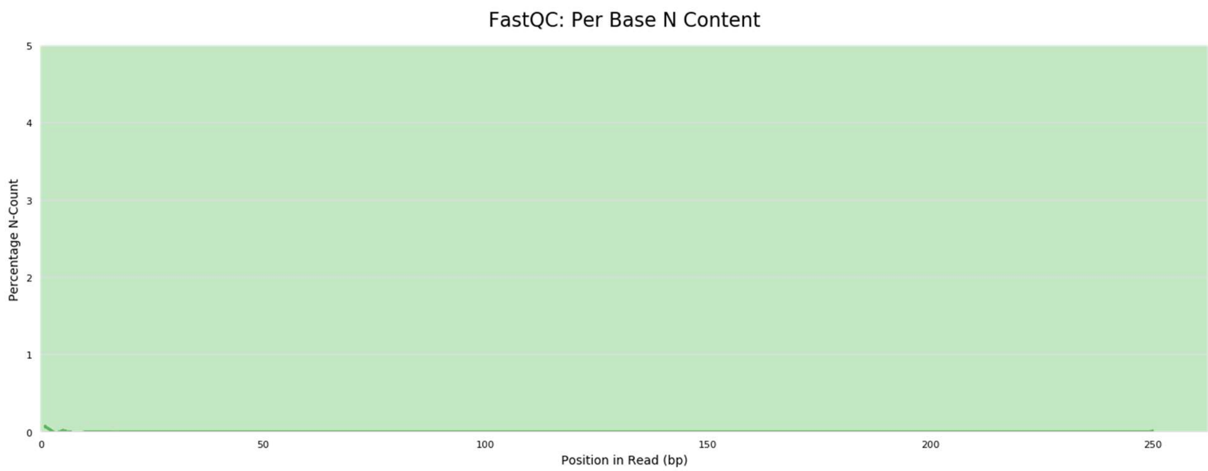

Figure 5. Per Base N Content. The graph shows the percentage of base calls at each position for which an N was called.

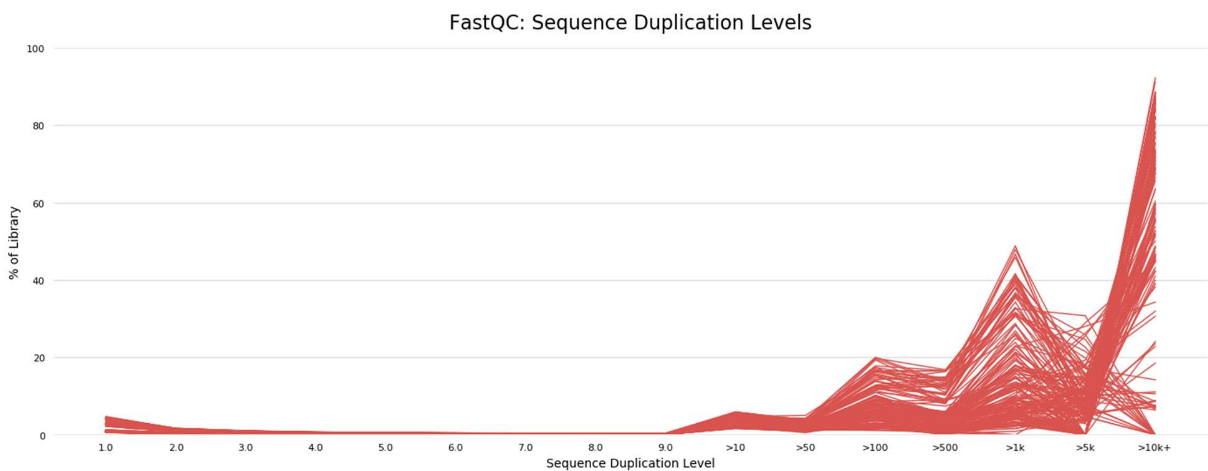

Figure 6. Sequence duplication levels. The relative level of duplication found for every sequence.

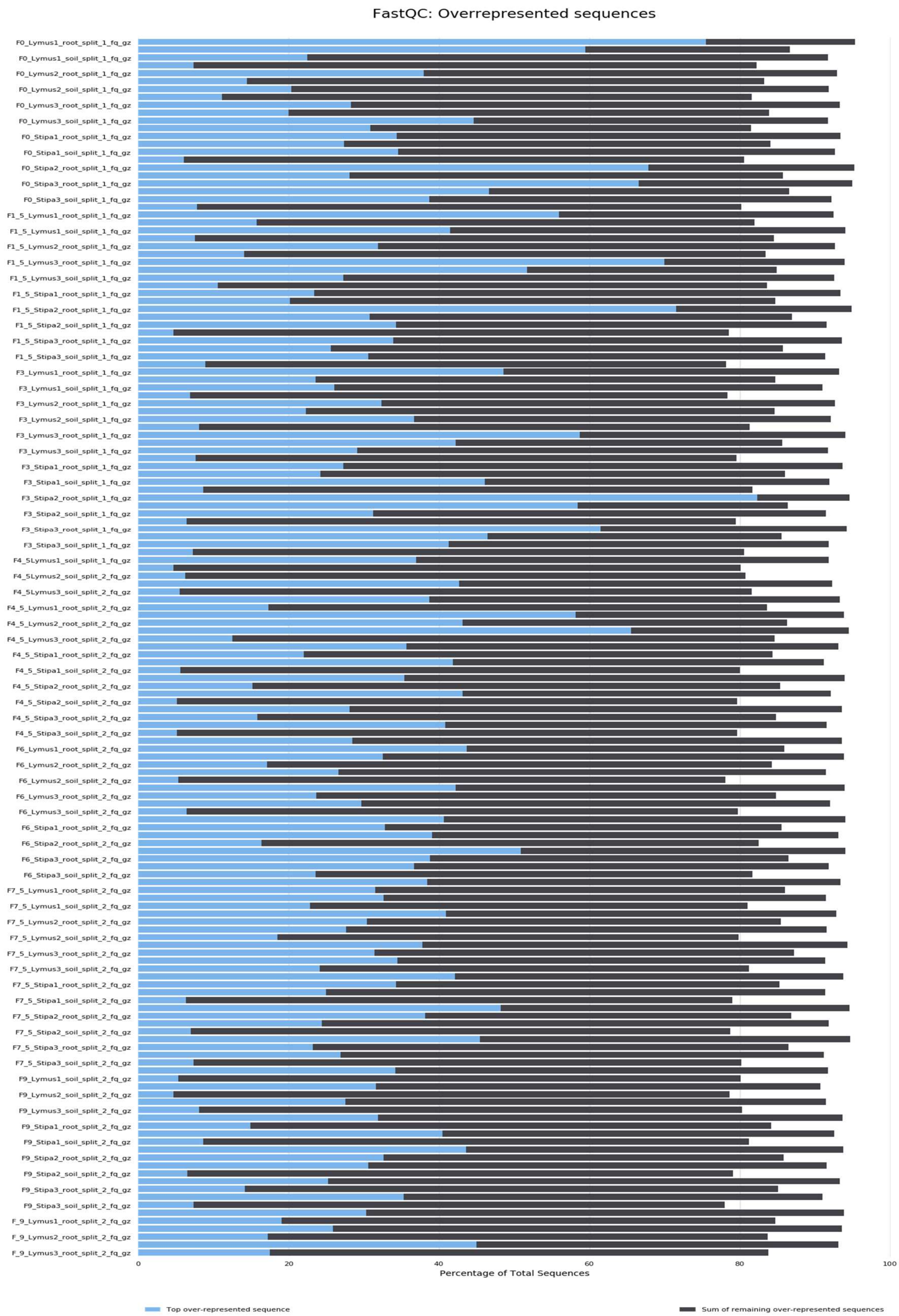

Figure 7. Over-represented sequences which is the total amount of overrepresented sequences found in each library. Sometimes, a single sequence may account for a large number of reads in a dataset. To show this, the bars are split into two: the first shows the overrepresented reads that come from the single most common sequence. The second shows the total count from all remaining overrepresented sequences.

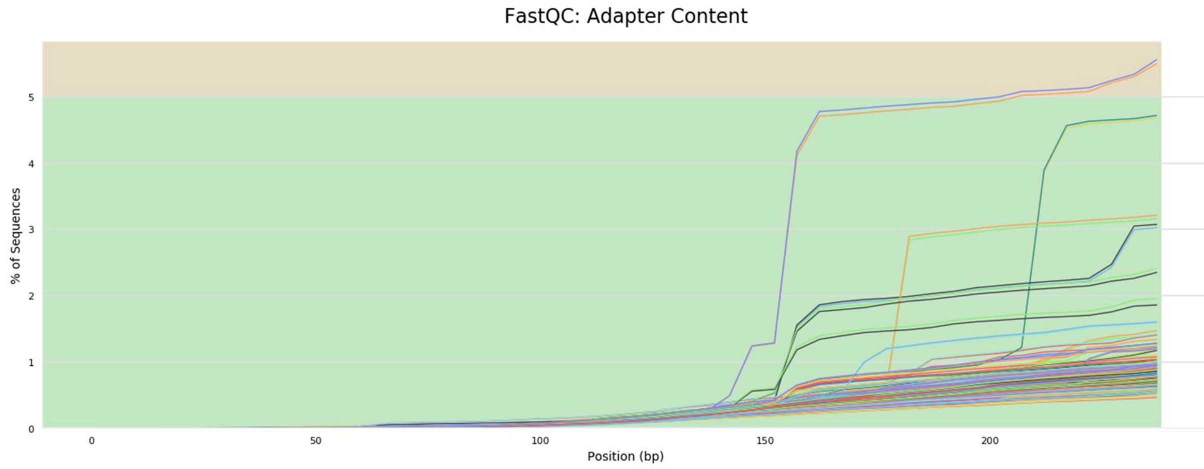

Figure 8. Adapter Content. The cumulative percentage count of the proportion of the library which has seen each of the adapter sequences at each position.