Version of Record: https://www.sciencedirect.com/science/article/pii/S1754504821000970
Manuscript_5e2e7705f90509a3430679f83cc62469

Effects of an introduced mustard, *Thlaspi arvense*, on soil fungal communities in subalpine meadows

Adam N. Trautwig[1,2], Mark A. Anthony[3] Serita D. Frey[3] and Kristina A. Stinson[1,2]

[1]*Department of Environmental Conservation, University of Massachusetts, 160 Holdsworth Way, Amherst, Massachusetts 01003, USA*; [2]*Rocky Mountain Biological Laboratory, 8000 County Road 317, Crested Butte, Colorado 81224, USA;*[3]*Department of Natural Resources and the Environment, University of New Hampshire, 56 College Road, Durham, New Hampshire 03824, USA*

Corresponding author: Adam Trautwig,

Postal address: University of Massachusetts, 160 Holdsworth Way, Amherst, Massachusetts, 01002, U.S.A.

Email: atrautwig@umass.edu

Tel: 631-332-3325

Fax: 413-545-4358

© 2021 published by Elsevier. This manuscript is made available under the Elsevier user license
https://www.elsevier.com/open-access/userlicense/1.0/

Abstract:

The subalpine meadows of the Rocky Mountains, USA, are at the advancing front of global change; however, little is known about the sensitivities of high-elevation soil fungal communities to ongoing ecological changes. Soil fungi are sensitive to abiotic and biotic environmental stressors, including climate change, soil disturbance, and the presence of introduced, non-native plants. Invasive plants in the Brassicaceae (mustard family) are known to alter fungal community structure, suppress arbuscular mycorrhizal fungi, and change their relationship with native plant hosts in forest ecosystems, but these phenomena have not been studied in the subalpine zone where non-native mustard plants are becoming established. Here, we investigated whether the presence of the introduced mustard plant, *Thlaspi arvense*, is associated with distinct properties of the whole fungal and arbuscular mycorrhizal fungal communities in subalpine meadow ecosystems. We observed clear differences in the composition, relative abundance of core taxa, and mean taxon relatedness of soil fungal communities in plots with *T. arvense* relative to those with only native vegetation. A suite of novel fungi were associated with *T. arvense*, and overall patterns of AMF phylogenetic diversity were drastically reduced in association with its presence. Our results suggest that *T. arvense* introduction impacts the soil fungal community, with potential implications for native plant communities and soil nutrient cycling in high elevation meadows of the Rocky Mountains.

Key Words: Brassicaceae, Plant introduction, ITS, 18s, Soil fungi, Arbuscular mycorrhizae, Phylogenetic diversity, Subalpine Meadows

Introduction:

Mountain ecosystems are home to unique biota as well as elevationally extreme populations of more widespread species (Marshall et al. 2011; Nakajima et al. 2014; Chmura et al. 2016; Donhauser and Frey 2018) and encompass relatively pristine subalpine communities that have historically escaped plant invasion (Averett et al. 2016). However, climate change and soil disturbance related to anthropogenic activity, in conjunction with the introduction of non-native plants, are increasingly threatening native biodiversity at high elevations (Pauchard et al. 2009).

Non-native plant species are known to alter soil fungal communities in North American forest ecosystems (e.g., Stinson et al. 2006; Wolfe et al. 2008; Anthony et al. 2017), and by extension can impact native plant communities (Alexander et al. 2016; Petitpierre et al. 2016), but this phenomenon has not been studied in subalpine meadow ecosystems. The impacts of non-native plant introduction on soil fungi could alter physical conditions in subalpine ecosystems and their native flora since soil microbial communities play a major role in the soil development and biogeochemical cycles governing plant establishment and distribution (Kivlin et al. 2013; Rudgers et al. 2014; Donhauser and Frey 2018).

The Eurasian wild mustard plant, *Thlaspi arvense* L. (field pennycress), is a widespread member of the Brassicaceae (mustard family), that is prevalent in disturbed sites across North America (Best and Mcintyre 1975). Increasingly, it is found in subalpine meadows where it has become more abundant in the last few decades (Chew 1975; Nakajima et al. 2013). The Brassicaceae family is comprised of largely non-mycorrhizal species that produce glucosinolates, a class of secondary compounds toxic to mycorrhizal fungi (Fahey et al. 2001; Roberts & Anderson 2001). *Thlaspi arvense* produces the glucosinolate sinigrin (Best and Mcintyre 1975; Chew 1977), which is known to disrupt native plant-mycorrhizal associations in forests of Northeastern North

America, when exuded by its invasive relative *Alliaria petiolata* (Renwick & Lopez 1999). While *A. petiolata* markedly transforms forest fungal communities (Anthony et al. 2017) and has particularly strong impacts on the composition and abundances of arbuscular mycorrhizal fungi (AMF; Barto et al. 2011), the effects of *T. arvense* on subalpine soil fungi is unknown. Moreover, which fungal taxa inhabit subalpine meadow ecosystems is poorly understood (Sundqvist et al. 2013; Siles and Margesin 2016; Hendershot et al. 2017) in comparison to that of other ecosystems (Freeman et al. 2009; Schmidt et al. 2012; Liu et al. 2015), which constrains our ability to predict responses of soil fungal communities to biological invasion in these unique landscapes.

The central aim of our study was to address the over-arching hypothesis that the non-native plant, *Thlaspi arvense,* alters the soil fungal community in subalpine meadows. Specifically, our objectives were to:

1. Test whether patches of meadow with *T. arvense* differ from those with normal vegetation in the overall composition of AMF and whole fungal communities.
2. Identify potential "indicator taxa" in the AMF and whole fungal communities that are either positively or negatively associated with *T. arvense*.
3. Compare the biodiversity of soil fungal taxa in patches with and without *T. arvense*.

Materials and Methods:

Site description

We examined the soil fungal communities in a subalpine meadow ecosystem located in proximity to the Rocky Mountain Biological Laboratory (RMBL; 38'57 °N, 106'59°W; approximately 2900 m), Colorado, USA. This area consists of typical native subalpine vegetation

dominated by forbs such as *Potentilla pulcherima, Potentilla hippiana*, *Delphinium barbeyi, Delphinium nelsonii, Lupinus sericeaus* and *Helianthella quinquenervis,* along with grasses such as *Festuca thurberii* (See CaraDonna and Inouye 2015). We established 54 0.25 m^2 study plots in six subalpine meadows where *Thlaspi arvense* was determined to be present, spanning about 400 m of elevation from ~2500-2900 m (*T. arvense* presence or absence was determined over the course of 20 minutes of searching). *Thlaspi arvense* was distributed in patches across the study sites (average density of 140.45 plants m^{-2}). Many *T. arvense* patches were associated with disturbances by animal (e.g., gopher, livestock) and human activities (e.g. hiking paths, recreational vehicle trails). We thus established 9 replicate 0.25 m^2 study plots (n = 3) in each meadow, separated by 2-5 m across three vegetation types/plot status: non-disturbed with dominant native vegetation (native; "Nveg"), disturbed due to animal or human activity without *T. arvense* present (disturbed; "D"), and disturbed plots with *T. arvense* present (invaded; "DTh"). In total, there were 54 plots (6 sites × 3 plot status × 3 replicates = 54 plots).

Soil sampling and processing

Soil samples were collected using a cylindrical corer (2 cm width × 10 cm depth). Nine samples were taken from each plot using a grid-design (8.3 cm apart) and were pooled together and homogenized into a single sample. Samples were kept on ice until returned to the laboratory where they were stored at 4°C. Within 48 hours, samples were sieved (<2 mm) to remove roots, rocks, and organic debris. Subsamples for molecular analysis were taken from the sieved soil and stored at -80°C. Soil pH and soil nutrient contents were determined on frozen samples stored at -10°C at the University of Massachusetts Soil and Plant Nutrient Testing facility (https://ag.umass.edu/services/soil-plant-nutrient-testing-laboratory).

115

116 *Vegetation survey*

117 Because plant species composition can sometimes affect the composition of soil fungal
118 communities, we also conducted a vegetation survey at each plot. We recorded all present
119 vegetation and counted the number of individuals per species at each plot. Field specimens were
120 collected in the field and pressed as needed for identification. Identities were determined using a
121 variety of online and printed field guides, with Weber & Wittmann (2012) as the final authority
122 for nomenclature.

123

124 *Fungal community characterization*

125 Total fungal and AMF community structure were assessed using *ITS2* and *18S* DNA
126 metabarcoding, respectively. DNA was extracted from soil (250 mg) using the Qiagen DNAeasy
127 kit (Qiagen, Hilden, Germany). The *ITS2* region was amplified using the primer combination
128 *fITS7-ITS4* (Ihrmark et al. 2012; White et al. 1990) to characterize the whole fungal community,
129 and the *18S* region was amplified using the primer combinations *NS31-AML2* (Simon et al. 1992;
130 Lee et al. 2008) to characterize fungi within the Glomeromycotina (AMF). Hereafter, we refer to
131 fungi identified by *ITS2* as 'fungi' and by *18S* as 'AMF'. PCR reactions, clean-up, and library
132 preparation were conducted as described by Anthony et al. (2020). Libraries were sequenced on
133 a MiSeq v2 (2 x 250 base pair chemistry) run and a MiSeq v3 (2 x 300 base pair chemistry) run
134 for *ITS2* and *18S*, respectively.

135

Sequences processing, clustering, and taxonomic assignment

First, we removed low quality reads (Median quality score < 38 and 37 for *18S* and *ITS2*, respectively), truncated single-end reads to 250 and 210 base pairs for *18S* and *ITS2* reads, respectively; and joined paired-end forward and reverse reads using QIIME2 2018.11 and established QIIME2 protocol for quality control (Bolyen et al. 2018). We extracted target *ITS2* reads from the *18S* and *5.8S* flanking regions (not *18S* reads due to QIIME2 recommendations), and clustered operational taxonomic units (OTUs) using *Deblur2* (Amir et al. 2017). All samples were rarified to the lowest sequencing depth using *Deblur2*. Sequences were clustered into OTUs using classifiers trained on the dynamically clustered UNITE fungal ITS database (Kõljalg et al. 2020) for the whole fungal community (accessed June 2018) and using the MaarjAM database (Öpik et al. 2010) for AMF. In addition, we performed BLAST searches on the top 100 most abundant OTUs that did not receive an assignment from the ITS2 targeted region -- representing 58.9 % of the total reads -- using the LCA algorithm in MEGAN6 (Huson et al. 2016). We removed singletons using base R. In total, there were 728 and 532 OTUs clustered in the ITS2 and 18S analyses, respectively. Whole fungal community composition was reported at the class level and Arbuscular mycorrhizal community composition was reported at the order level, according to available reference databases. Taxonomic bar plots were constructed for sequences obtained to visualize differences in the relative abundances of taxa across the different types of plot status.

Statistical analysis

We conducted all analyses in R 4.0.2, unless otherwise noted.

158 To determine whether fungal community composition in soils where *T. arvense* is present differs
159 from fungal community composition where it is absent, we used non-metric multidimensional
160 scaling (NMDS) and PERMANOVA. In addition to fungal communities, we applied these
161 analyses to edaphic variables and vegetation as well. We used the metaMDS function to
162 visualize variation in the whole fungal and AMF communities (Bray-Curtis dissimilarity). To
163 test for differences across the plot status categories, we ran PERMANOVA through the 'adonis'
164 function in *vegan* (999 permutations), with plot status as the main effect, nested within site.
165 Although the plant communities were similar at each study location, we chose a nested model to
166 test for differences among patches with different vegetation status categories (native vegetation,
167 disturbance without *T. arvense*, and disturbance with *T. arvense*), while accounting for potential
168 environmental heterogeneity across the six study sites.

169 We used an indicator species analysis to determine which whole fungal community and AMF
170 OTUs may be positively or negatively associated with *T. arvense.* Indicator species analysis was
171 performed using the *indicspecies* package (De Caceres and Legendre 2009) with plot status as
172 the main effect and the frequency of each OTU as the response variable. Separate indicator
173 species analyses were performed for the whole fungal community and for AMF.

174 To determine if biodiversity of soil fungal taxa varied with *T. arvense* presence, we quantified
175 phylogenetic signal in AMF communities through community phylogenetic signal analysis.
176 Phylogenetic signal was assessed using the *picante* package to determine whether the distribution
177 of AMF was more or less phylogenetically related than would be expected by chance across plot
178 status. This analysis was constrained to the AMF community since *18S* sequences are
179 phylogenetically conserved whereas *ITS2* reads are not useful for this type of analysis
180 (Stockinger et al. 2010). Following Egan et al. (2017) for tree construction and phylogenetic

signal analysis, we mid rooted *18S* phylogenetic trees using default functions in the *picante* package and assessed phylogenetic signal using independent swaps for null model simulations after 999 permutations. We computed standardized effect sizes (SES) as $X_{Observed} - mean(X_{Null})/SD(X_{Null})$. Positive SES values indicate a higher level of phylogenetic clustering than would be expected by chance and negative SES values indicate a lower level of phylogenetic clustering than would be expected by chance. SES of mean phylogenetic distance (MPD) and SES of mean nearest-taxon distance (MNTD) was computed (Kembel et al. 2010). We tested for effects of plot status on SES values using a nested ANOVA model with plot status as the main effect, nested in site, specifying the SES values as the response variable. We also compared models to determine which variables explained the most variation within MPD and MNTD data sets.

To account for potential effects of local edaphic and vegetation conditions, we used ENVIFIT with 999 permutations to determine whether the NMDS coordinates for AMF and whole fungal communities were correlated with specific soil nutrients and specific plant species.

Results:

Effects of Thlaspi arvense on fungal community composition

Fungal community composition varied by plot status nested by site for AMF ($p = 0.014$) but not the whole fungal community ($p = 0.113$), and there was a significant effect of site in both models (Table 1, Figure 1). The taxonomic structure of the AMF community also varied by plot status. For AMF, the families Aculosporaceae and Glomeraceae had higher relative abundances in disturbed and invaded vegetation types relative to intact native vegetation (Figure 2). One unidentified family in the Glomerales had lower relative abundance in the invaded plots than in

plots with the other vegetation types. Taxonomic structure did not vary by vegetation type for the whole fungal community.

Positive and negative whole fungal and AMF species (OTU) level associations with T. arvense

A suite of fungal OTUs were significant indicator species of native and disturbed versus invaded plots (Table 2). Not all of the OTUs found to be indicators of specific plot status had matches in GenBank and as such more research is necessary to determine the implications of these OTUs. Seven OTUs were associated with native vegetation sites, with an unidentified *Glomus* sp. being most indicative. Twice as many taxa were significant indicators of the disturbed sites, with an unidentified Leotiomyctes and Mortierella spp being most indicative. Seven more OTUs were associated with disturbed and *T. arvense* plots, with an uncultured Glomeraceae being most representative. Several of these OTUs were indicators of more than one plot status, but there was no overlap between the indicators found across native vegetation and disturbed with *T. arvense* present plots, which suggests that these plots are the most dissimilar.

Phylogenetic signal in AMF communities with and without T. arvense

AMF communities in the plots with *T. arvense* were more phylogenetically disparate than communities in the native vegetation plots (Figure 3). Across plot status, SES MPD ($p=0.02$) and SES MNTD ($p=0.007$) were different. Models with plot status and site explained about a quarter of the variation in SES MPD ($F_{(8,48)}=3.29$, $p=0.004$, $r^2=0.25$) and SES MNTD ($F_{(8,48)}=3.363$, $p=0.004$, $r^2=0.25$) and significantly varied from null models ($p=0.018$ and $p=0.032$, respectively).

225

226 *Edaphic features and fungal communities*

227 Edaphic features did not vary by plot status nested by site (Supplemental Table 1; Supplemental
228 Figure 1). Several edaphic features were significantly correlated with both whole fungal
229 community and AMF community composition in the vector analysis (Supplemental Table 2). Of
230 the edaphic features included in this analysis, soil pH (r^2=0.59, p=0.001), exchangeable acidity
231 (Ea) (r^2=0.52, p=0.001), and sulfur (S) (r^2=0.46, p=0.001) were most highly correlated with
232 whole fungal community composition. Arbuscular mycorrhizal community composition was
233 associated with cation exchange capacity (CEC) (r^2=0.52, p=0.001), Mg (r^2=0.47, p=0.001), and
234 Ca (r^2=0.45, p=0.001).

235

236 *Relationships between fungal and plant communities*

237 Plant community composition varied by plot status nested by site (Supplemental Table 1;
238 Supplemental Figure 1). There were also some associations between plant species and fungal
239 community composition (Supplemental Table 3). In both communities *Bromus* sp. (r^2=0.14,
240 p=0.030 and r^2=0.20, p=0.003, whole fungal and AMF community, respectively) and an
241 unidentified herbaceous plant species (r^2=0.12, p=0.044 and r^2=0.12, p=0.036, whole fungal and
242 AMF community, respectively) were significantly correlated with fungal community
243 composition. In whole fungal communities, *Poa annua* (r^2=0.45, p=0.001), *Cirsum* sp. (r^2=0.28,
244 p=0.001), *Potentilla pulcherrima* (r^2=0.25, p=0.004), and *Phleum pratense* (r^2=0.16, p=0.004)
245 were also correlated with fungal community composition. *Erigeron* sp. (r^2=0.14, p=0.009) and an

246 additional unidentified herbaceous plant (r^2=0.09, p=0.038) were correlated with AMF
247 community composition.

248

249 **Discussion:**

250 The main goal of our study was to test the hypothesis that the non-native plant, *Thlaspi arvense,*
251 alters the soil fungal community in subalpine meadows, given that its close relative, *A. petiolata*
252 is known to alter soil biota in North American forest soils (Anthony et al. 2017). In support of
253 this prediction, we found that patches of subalpine meadow with *T. arvense* were distinct in the
254 overall composition of AMF communities, that there were some specific "indicator taxa" in both
255 the AMF and whole fungal communities that are either positively or negatively associated with
256 *T. arvense*, and that soils with *T. arvense* present were less phylogenetically clustered in the
257 AMF community. We also noted some possible contributions of edaphic and vegetation
258 properties to variation among soil fungal communities and evidence for heterogeneity among the
259 study sites. Below we discuss our findings in the context of the prior literature and consider the
260 implications for future invasion by *T. arvense* into high altitude plant communities.

261

262 *Effects of T. arvense on fungal community composition*

263 Soil microbial communities are recognized as playing a major role in soil development as well as
264 biogeochemical cycles governing plant establishment, with some fungal associates buffering the
265 effects of temperature and available soil moisture on plant associates (Kivlin et al. 2013; Rudgers
266 et al. 2014; Donhauser and Frey 2018). We found evidence for effects of plot status (i.e.,
267 presence of non-native *T. arvense*) on AMF community composition, suggesting that *T. arvense*

268 can influence the composition of the arbuscular mychorrhizal community and by extension
269 native subalpine plants that rely on certain AMF partners. Our findings are consistent with prior
270 work showing that another invasive mustard, *A. petiolata,* alters AMF and associted plant
271 communities in forest ecosystems (Stinson et al., 2006; Callaway et al., 2008; Barto et al., 2011;
272 Anthony et al., 2017). Our work also contributes to a growing body of work suggesting that other
273 invasive plant taxa affect native plant communities via changes in the soil biota (Callaway et al.
274 2004; Trautwig et al. 2016; Řezáčová et al. 2021).

275 Furthermore, shifts in dominant fungal trophic guilds in response to Brassiceace invasions which
276 favor free-living decomposer fungi over mycorrhizal taxa is associated with reductions in soil C
277 contents (Anthony et al. 2017). Therefore, in some sites, increasing abundances of *T. arvense*
278 could have a substantial effect on ecosystem processes.

279

280 *Positive and negative whole fungal and AMF species (OTU) level associations with T. arvense*

281 At broad taxonomic levels, we found that the composition of fungal taxonomic groups varied
282 with *T. arvense* presence or absence. Several OTUs were identified through indicator species
283 analysis to vary significantly across plot status and elevation. We found that 60% of *T. arvense*-
284 associated indicator species were entirely absent from the native vegetation plots, providing
285 some insight into which taxa are most sensitive to *T. arvense*. Various members of the AMF
286 genus, *Glomus*, were indicator species of plot status. These biotrophic taxa require a host plant,
287 and the presence of *Glomus* OTUs in each plot type is not unusual due to the diversity and
288 functional divergence present across this genus (Simon et al. 1993). Spore germination in some
289 *Glomus* species is suppressed in the presence of *A. petiolata* (Stinson et al. 2006), and both *A.*

petiolata and *T. arvense* are considered non-mycorrhizal themselves. One possibility is that *T. arvense* might suppress a number of *Glomus* species only found in association with native vegetation but that some *Glomus* species are less sensitive and may even respond positively to new niche opportunities in the presence of this non-native mustard (Anthony et al. 2020). More insight into *Glomus* biology are necessary to definitively state what makes specific OTUs in the *Glomus* complex successful in this context.

It is interesting to also note that more OTUs were associated with disturbed plot status than either native or disturbed with *T. arvense*, across a range of genera. Previous work has shown that local taxonomic heterogeneity can increase with small-scale disturbances (Morris et al. 2007), which may explain why there were more indicator taxa in the disturbance plots if there are increased opportunities for niche partitioning. Similar to shifts from a mycorrhizal-dominated to a saprotroph-dominated community in the presence of invasive *A. petiolata* (Anthony et al. 2017), the disturbance indicator species in our study were predominantly saprotrophs and therefore may indicate some differences between disturbed and undisturrbed sites in local nutrient cycling (Dighton 2007).

Phylogenetic signal in AMF communities with and without T. arvense

Whether and how invasive plants affect the phylogenetic diversity of soil biota remains relatively under-explored, but prior work suggests some invasions can increase phylogenetic diversity, possibly due to co-invasions of fungal and plant taxa (Lekberg et al. 2013). Co-invasion involving AMF has, similarly, been rarely investigated (Aslani et al. 2019), however lower levels of phylogenetic relatedness in meadows where *T. arvense* is present may indicate subsequent

312 invasions. Here we found that patches where *T. arvense* was present had a lower degree of
313 relatedness compared to non-disturbed patches where the invader is absent. This finding
314 suggests that overall AMF biodiversity could be shifting away from high-elevation specialists
315 and towards generalists with *T. arvense* introductions as fungal communities re-assemble.

316

317 *Edaphic features, plants, and fungal communities effect site-to-site heterogeneity*

318 Extensive heterogeneity in edaphic conditions and plant communities were present across sites
319 with strong correlations between fungal community composition and certain edaphic properties
320 and plant species. These results are consistent with other work showing strong effects of
321 environmental filtering on fungal communities (Kivlin et al. 2014, Glassman et al. 2015), and
322 were independent of *T. arvense* presence. Environmental conditions often vary over short
323 distances in the subalpine zone (Donhauser and Frey 2018), and many fungi are thought to be
324 locally endemic in these ecosystems (Noffsinger et al. 2020). Across all sites, soil chemistry
325 variables appeared to be important filters associated with variation in fungal community
326 composition, as indicated by the high number of significant edaphic correlations. Several edaphic
327 variables, including CEC, and Ca and S concentrations, have either general implications for
328 microbial biomass or specific implications for *Thlaspi*-microbial interactions (Fahey et al. 2001;
329 Casazza et al. 2017). Here we show significant variation in the fungal and plant communities as
330 well as edaphic variables and plant species associated with fungal communities, which suggests
331 that there may be landscape-scale effects of plant invasion even across a heterogeneous
332 environment like the subalpine zone.

333

Conclusion:

As a result of environmental changes and the introduction of non-native species around the globe, novel ecological interactions are occurring among organisms that have not historically co-existed (Pauchard et al. 2009; Averett et al. 2016), especially in the more pristine habitats of subalpine meadows. These new interactions raise many ecological questions, including whether and how native species re-assemble after invasion. Our research addresses invasive plants and their effects on native fungal communities of North American subalpine meadows. We found that the widespread introduced plant, *Thlaspi arvense* significantly altered AMF communities in spite of significant heterogeneity in AMF and the whole fungal community across sites. Several fungal indicator OTUs were found to be associated with different plot status and may represent key components of community construction in the presence and absence of *T. arvense*. In addition, the presence of *T. arvense* resulted in lower levels of phylogenetic diversity than would have been expected in AMF communities, suggesting a shift from a more diverse community comprised of various specialist taxa to a more homogenous one comprised of generalists. The shifts in fungal community composition and diversity in the presence of *T. arvense* are likely responses to the unique phytochemistry in this non-native mustard plant, as has been shown for invasive relatives in other ecosystems (Stinson et al. 2006). As such, our work supports a growing body of literature on the importance of "novel weapons" by which some invasive plants disrupt native soil biota, with implications for plant community and soil nutrient cycling dynamics. Overall, we provide evidence that early detection and rapid response to increasing abundances of *T. arvense* may be warranted in the subalpine zone.

Acknowledgements:

This work was funded in part by a U.S. Department of Defense Strategic Environmental Research and Development Program (SERDP) Grant (NRC2326) to KAS and SDF. Views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of Defense position or decision unless so designated by other official documentation. Rick Williams was integral in identification of plant specimens at the Rocky Mountain Biological Laboratory. Stephanie Kivlin, Kristen DeAngelis, and Lauren Carley offered input on experimental design and statistical analysis. Mel Knorr offered technical expertise and assistance in laboratory procedures. Elsa Cousins and Michelle Jackson provided feedback on early drafts of this paper.

References:

Amir, Amnon, Daniel McDonald, Jose A. Navas-Molina, Evguenia Kopylova, James T. Morton, Zhenjiang Zech Xu, Eric P. Kightley et al. "Deblur rapidly resolves single-nucleotide community sequence patterns." *MSystems* 2, no. 2 (2017).

Anthony, M. A., Frey, S. D., & Stinson, K. A. (2017). Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. *Ecosphere*, *8*(9).

Anthony, M.A., Stinson, K.A., Moore, J.A.M., Frey, S.D. (2020) Plant invasion impacts on fungal community structure and function depend on soil warming and nitrogen enrichment. *Oecologia* (in press)

Averett, J. P., McCune, B., Parks, C. G., Naylor, B. J., DelCurto, T., & Mata-Gonzalez, R. (2016). Non-native plant invasion along elevation and canopy closure gradients in a middle Rocky Mountain ecosystem. *PLoS One*, 11(1).

Barto, E. K., Antunes, P. M., Stinson, K., Koch, A. M., Klironomos, J. N., & Cipollini, D. (2011). Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. *Biological Invasions*, *13*(12), 2755-2762.

Best, K. F., & McIntyre, G. I. (1975). The biology of Canadian weeds: 9. Thlaspi arvense L. *Canadian Journal of Plant Science*, *55*(1), 279-292.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C., Al-Ghalith, G. A., ... & Bai, Y. (2018). *QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science* (No. e27295v1).

Callaway, R. M., Cipollini, D., Barto, K., Thelen, G. C., Hallett, S. G., Prati, D., ... and Klironomos, J. (2008). Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*, 89(4): 1043-1055.

CaraDonna, P. J., & Inouye, D. W. (2015). Phenological responses to climate change do not exhibit phylogenetic signal in a subalpine plant community. *Ecology*, *96*(2), 355-361.

Casazza, G., Lumini, E., Ercole, E., Dovana, F., Guerrina, M., Arnulfo, A., ... & Mucciarelli, M. (2017). The abundance and diversity of arbuscular mycorrhizal fungi are linked to the soil chemistry of screes and to slope in the Alpic paleo-endemic Berardia subacaulis. *PloS one*, *12*(2).

Chew, F. S. (1975). Coevolution of pierid butterflies and their cruciferous foodplants. *Oecologia*, *20*(2), 117-127.

Chew, F. S. (1977). Coevolution of pierid butterflies and their cruciferous foodplants. II. The distribution of eggs on potential foodplants. *Evolution*, *31*(3), 568-579.

Chmura, H. E., Wey, T. W., & Blumstein, D. T. (2016). Assessing the sensitivity of foraging and vigilance to internal state and environmental variables in yellow-bellied marmots (Marmota flaviventris). *Behavioral ecology and sociobiology*, *70*(11), 1901-1910.

De Caceres, M., and Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology* 90, 3566–3574.

Dighton, J. (2007). 16 Nutrient Cycling by Saprotrophic Fungi in Terrestrial Habitats. *Environmental and microbial relationships*, *4*, 287.

Donhauser, J., & Frey, B. (2018). Alpine soil microbial ecology in a changing world. *FEMS Microbiology Ecology*.

Duchesneau, K., Golemiec, A., Colautti, R. I., & Antunes, P. M. (2021). Functional shifts of soil microbial communities associated with Alliaria petiolata invasion. *Pedobiologia*, *84*, 150700

Egan, C. P., Callaway, R. M., Hart, M. M., Pither, J., & Klironomos, J. (2017). Phylogenetic structure of arbuscular mycorrhizal fungal communities along an elevation gradient. *Mycorrhiza*, *27*(3), 273-282.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry, 56*(1), 5-51.

Freeman, K. R., Martin, A. P., Karki, D., Lynch, R. C., Mitter, M. S., Meyer, A. F., ... & Schmidt, S. K. (2009). Evidence that chytrids dominate fungal communities in high-elevation soils. *Proceedings of the National Academy of Sciences*, pnas-0907303106.

Funk, J., Saunders, S., Sanford, T., Easley, T., and Markham, A., (2014). *Rocky Mountain forests at risk: Confronting climate-driven impacts from insects, wildfires, heat, and drought. Report from the Union of Concerned Scientists and the Rocky Mountain Climate Organization*. Cambridge, MA: Union of Concerned Scientists.

Glassman, S. I., Peay, K. G., Talbot, J. M., Smith, D. P., Chung, J. A., Taylor, J. W., ... & Bruns, T. D. (2015). A continental view of pine-associated ectomycorrhizal fungal spore banks: a quiescent functional guild with a strong biogeographic pattern. *New phytologist*, *205*(4), 1619-1631.

438 Hendershot, J. N., Read, Q. D., Henning, J. A., Sanders, N. J., & Classen, A. T. (2017).
439 Consistently inconsistent drivers of microbial diversity and abundance at macroecological scales.
440 *Ecology*, *98*(7), 1757-1763.

441 Hill, M. O. (1973). Diversity and evenness: a unifying notation and its consequences. *Ecology,*
442 *54*(2), 427-432.

443 Huson, D. H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., ... & Tappu, R. (2016).
444 MEGAN community edition-interactive exploration and analysis of large-scale microbiome
445 sequencing data. PLoS computational biology, 12(6).

446 Ihrmark, K., Bödeker, I., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., ... &
447 Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region–evaluation by 454-
448 sequencing of artificial and natural communities. *FEMS microbiology ecology*, *82*(3), 666-677.

449 Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., ... &
450 Webb, C. O. (2010). Picante: R tools for integrating phylogenies and
451 ecology. *Bioinformatics*, *26*(11), 1463-1464.

452 Kivlin, S. N., Emery, S. M., & Rudgers, J. A. (2013). Fungal symbionts alter plant responses to
453 global change. *American Journal of Botany*, *100*(7), 1445-1457.

454 Kivlin, S. N., Winston, G. C., Goulden, M. L., & Treseder, K. K. (2014). Environmental filtering
455 affects soil fungal community composition more than dispersal limitation at regional
456 scales. *Fungal Ecology*, *12*, 14-25.

Kõljalg, U., Nilsson, H. R., Schigel, D., Tedersoo, L., Larsson, K. H., May, T. W., ... & Abarenkov, K. (2020). The taxon hypothesis paradigm—On the unambiguous detection and communication of taxa. *Microorganisms*, *8*(12), 1910.

Lee, J., Lee, S., & Young, J. P. W. (2008). Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS microbiology ecology*, *65*(2), 339-349.

García de León, D., Davison, J., Moora, M., Öpik, M., Feng, H., Hiiesalu, I., ... & Sepp, S. K. (2018). Anthropogenic disturbance equalizes diversity levels in arbuscular mycorrhizal fungal communities. *Global Change Biology*, *24*(6), 2649-2659.

Liu, L., Hart, M. M., Zhang, J., Cai, X., Gai, J., Christie, P., ... & Klironomos, J. N. (2015). Altitudinal distribution patterns of AM fungal assemblages in a Tibetan alpine grassland. *FEMS microbiology ecology*, *91*(7).

Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial ecology in health and disease*, *26*(1), 27663.

Marshall, S. J., White, E. C., Demuth, M. N., Bolch, T., Wheate, R., Menounos, B., ... & Shea, J. M. (2011). Glacier water resources on the eastern slopes of the Canadian Rocky Mountains. *Canadian Water Resources Journal*, *36*(2), 109-134.

Morris, S. J., Friese, C. F., & Allen, M. F. (2007). Disturbance in natural ecosystems: scaling from fungal diversity to ecosystem functioning. *Environmental and microbial relationships. The Mycota*, *3*, 1-45.

Nakajima, M., Boggs, C. L., Bailey, S., Reithel, J., & Paape, T. (2013). Fitness costs of butterfly oviposition on a lethal non-native plant in a mixed native and non-native plant community. *Oecologia*, *172*(3), 823-832.

Nakajima, M., Boggs, C. L., Chew, F. S., Cummings, A., & Bowsher, J. H. (2014). Dynamics and structure of a native Pieris population in the presence of a non-native, toxic larval host plant. *The Journal of the Lepidopterists' Society*, *68*(3), 175-184.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., ... & Kennedy, P. G. (2016). FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241-248.

Nilsson, R. H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., & Tedersoo, L. (2019). Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology, 17*(2), 95-109.

Noffsinger, C., Cripps, C. L., & Horak, E. (2020). A 200-year history of arctic and alpine fungi in North America: Early sailing expeditions to the molecular era. *Arctic, Antarctic, and Alpine Research*, *52*(1), 323-340.

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J. M., ... & Zobel, M. (2010). The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist*, *188*(1), 223-241.

Pauchard, A., Kueffer, C., Dietz, H., Daehler, C. C., Alexander, J., Edwards, P. J., ... & Jakobs, G. (2009). Ain't no mountain high enough: plant invasions reaching new elevations. *Frontiers in Ecology and the Environment*, *7*(9), 479-486.

Renwick, J. A. A., & Lopez, K. (1999). Experience-based food consumption by larvae of Pieris rapae: addiction to glucosinolates?. In Proceedings of the 10th International Symposium on Insect-Plant Relationships (pp. 51-58). Springer, Dordrecht.

Řezáčová, V., Řezáč, M., Gryndler, M., Hršelová, H., Gryndlerová, H., & Michalová, T. (2021). Plant invasion alters community structure and decreases diversity of arbuscular mycorrhizal fungal communities. *Applied Soil Ecology*, *167*, 104039

Roberts, K. J., & Anderson, R. C. (2001). Effect of garlic mustard [Alliaria petiolata (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *The American Midland Naturalist*, *146*(1), 146-152.

Roswell, M., Dushoff, J., & Winfree, R. (2021). A conceptual guide to measuring species diversity. *Oikos*, *130*(3), 321-338.

Rudgers, J. A., Kivlin, S. N., Whitney, K. D., Price, M. V., Waser, N. M., & Harte, J. (2014). Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming. *Ecology*, *95*(7), 1918-1928.

Schmidt, S. K., Naff, C. S., & Lynch, R. C. (2012). Fungal communities at the edge: ecological lessons from high alpine fungi. *Fungal Ecology*, *5*(4), 443-452.

Siles, J. A., & Margesin, R. (2016). Abundance and diversity of bacterial, archaeal, and fungal communities along an altitudinal gradient in alpine forest soils: what are the driving factors?. *Microbial ecology*, *72*(1), 207-220.

Simon, L., Lalonde, M., & Bruns, T. D. (1992). Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied and environmental microbiology*, *58*(1), 291-295.

Simon, L., Bousquet, J., Lévesque, R. C., & Lalonde, M. (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature*, *363*(6424), 67-69.

Stockinger, H., Krüger, M., & Schüßler, A. (2010). DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist*, *187*(2), 461-474.

Stinson, K. A., Campbell, S. A., Powell, J. R., Wolfe, B. E., Callaway, R. M., Thelen, G. C., ... & Klironomos, J. N. (2006). Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS biology*, *4*(5), e140

Sundqvist, M. K., Sanders, N. J., & Wardle, D. A. (2013). Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 261-280.

Trautwig, A. N., Eckhardt, L. G., Loewenstein, N. J., Hoeksema, J. D., Carter, E. A., & Nadel, R. L. (2017). Cogongrass (Imperata cylindrica) affects above-and belowground processes in commercial loblolly pine (Pinus taeda) stands. *Forest Science*, *63*(1), 10-16.

Weber, W. A., & Wittmann, R. C. (2012). Colorado Flora: Western Slope, A Field Guide to the Vascular Plants. University Press of Colorado.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, *18*(1), 315-322.

Wolfe, B. E., Rodgers, V. L., Stinson, K. A., & Pringle, A. (2008). The invasive plant Alliaria petiolata (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology*, *96*(4), 777-783.

Figure Legends:

Figure 1. Fungal communities are represented by Bray-Curtis distribution across sites. Points represent centroids and error bars represent standard error. Soil nutrient vectors were included in both NMDS plots using 999 permutations. A) Whole fungal community B) Arbuscular mycorrhizal fungal community

Figure 2. Taxonomic bar plots were constructed for sequences obtained. A) Whole fungal community composition was reported at the class level to visualize differences between diversity between plot status. B) Arbuscular mycorrhizal community composition was reported at the order level in order to add context within the existing body of published research and are shown here divided by plot status.

Figure 3. Arbuscular mycorrhizal communities were analyzed through phylogenetic signal analysis. Standard effect sizes (SES) are presented for A) mean phylogenetic distance (MPD) between plot status and B) mean nearest taxon distance (MNTD) for sequences amplified from the 18s region. Analysis of variance is presented for differences among plot status in SES MPD and SES MNTD, respectively. Significant differences determined at $\alpha=0.05$ level are represented by Tukey variables.

562 Table 1. PERMANOVA for variables at 999 permutations across whole fungal community and
563 arbuscular mycorrhizal fungi communities. Variables that were significant at $\alpha = 0.05$ are
564 presented as bolded.

565

Whole Fungal Community

	F-value	R^2	P-value
Vegetation Status nested by Site	0.987	0.042	0.113

Arbuscular Mycorrhizal Fungi Community

	F-value	R^2	P-value
Vegetation Status nested by Site	**1.279**	**0.048**	**0.014**

566

567

568

569

570

571

572

573

574 Table 2. Both whole fungal community and arbuscular mycorrhizal fungal community were subjected to an indicator species analysis.

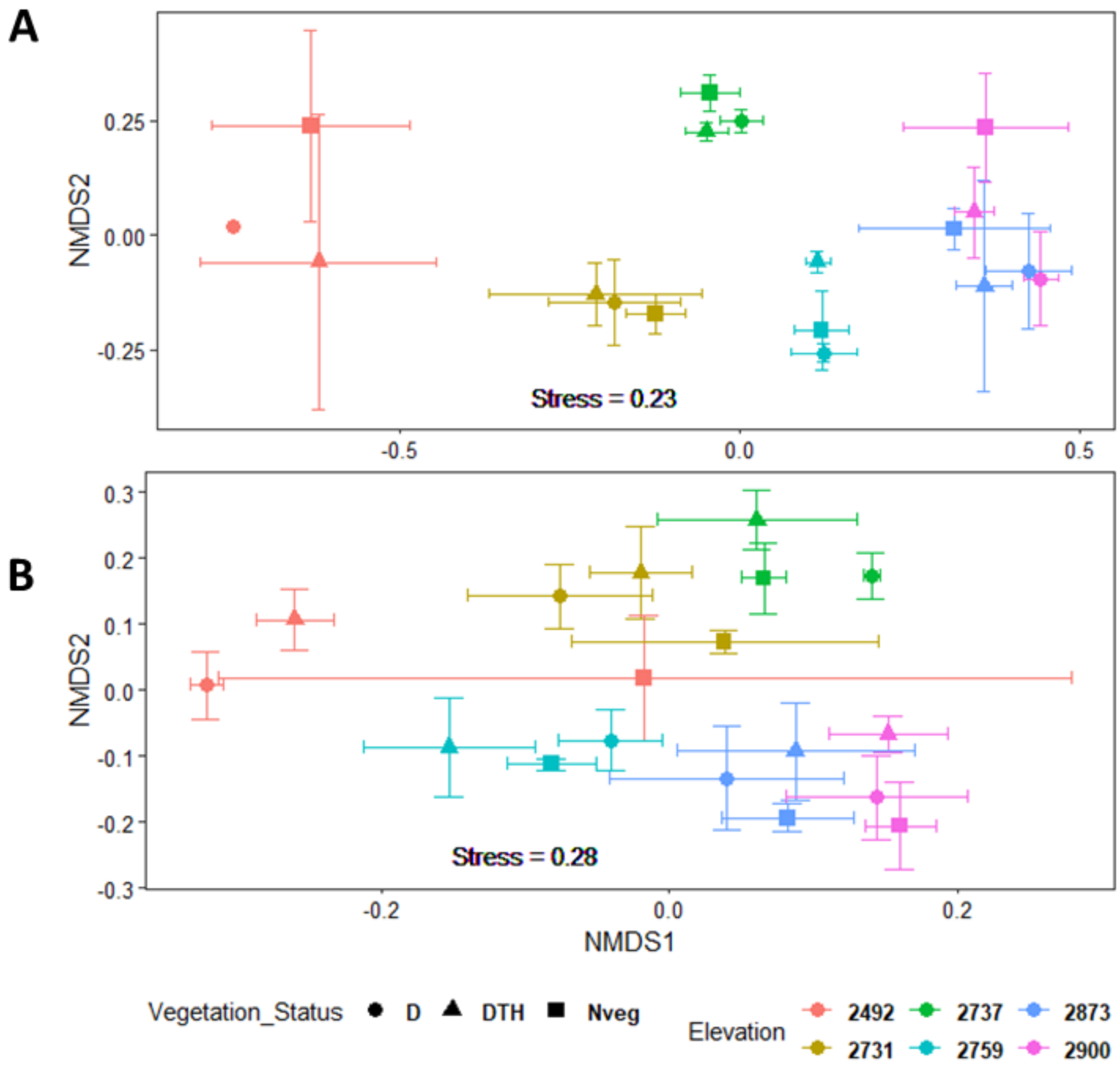
575 OTUs that were indicated to be significant at $\alpha = 0.05$ in a specific treatment were manually identified to the closest genus level match

576 in BLAST. Relative abundance of OTU and indicator species analysis statistic are presented alongside identity.

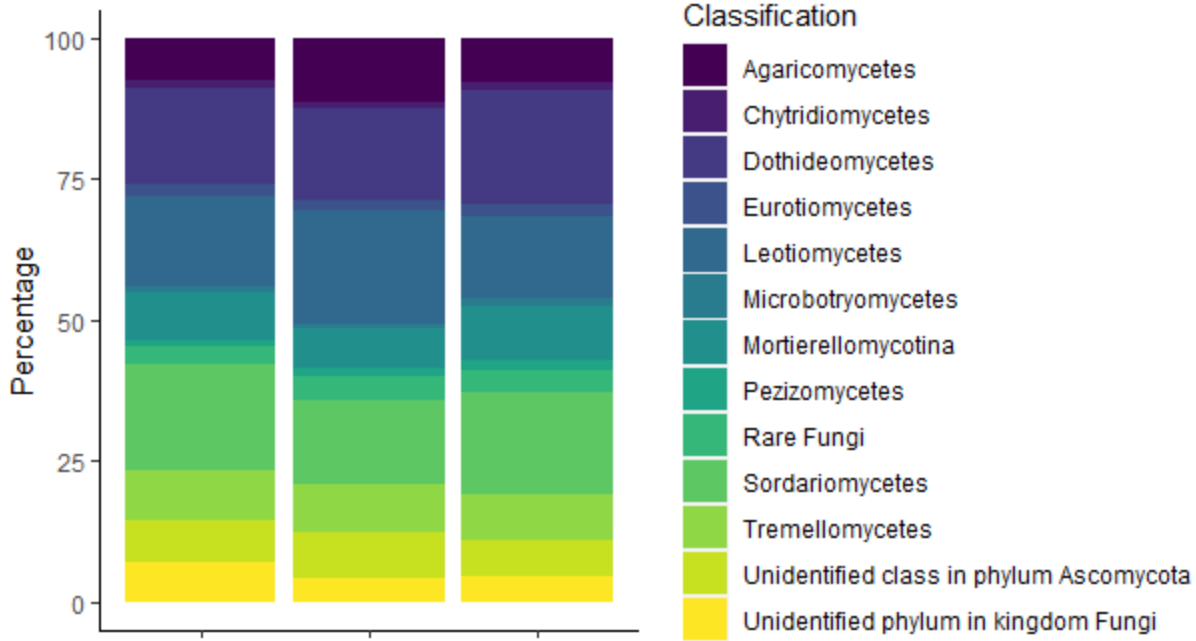
577

Normal Vegetation			**Disturbed**			**Disturbed+*Thlaspi arvense***		
Identity	*Relative Abundance*	*ISA Statistic*	*Identity*	*Relative Abundance*	*ISA Statistic*	*Identity*	*Relative Abundance*	*ISA Statistic*
Pleosporales	0.0051	0.480	Glomus	0.0009	0.471	Uncultured Glomeraceous Fungi	0.0238	0.848
Uncultured Glomeraceous Fungi	0.0018	0.464	Lambertella	0.0063	0.467	Uncultured Environmental Fungi	0.0028	0.527
Apodus	0.0054	0.584	Leotiomycetes	0.0097	0.797	Leotiomycetes	0.0097	0.797
Helotiales	0.0010	0.459	Mortierella	0.0106	0.775	Mortierella	0.0106	0.775
Glomus	0.0045	0.721	Glomus	0.0045	0.721	Uncultured Environmental Fungi	0.0087	0.625
Glomus	0.0055	0.641	Glomus	0.0055	0.641	Helotiales/Tetracladium	0.0016	0.490
Diversisporales	0.0116	0.506	Diversisporales	0.0116	0.506	Trichocladium/Pleotrichocladium	0.0038	0.600
			Sporormiella	0.0010	0.516			
			Helotiales	0.0013	0.490			
			Claroideoglomus	0.0010	0.480			
			Paraphaeosphaeria	0.0005	0.447			
			Mycena	0.0005	0.447			
			Phoma/Cadophora	0.0005	0.447			
			Trichocladium/Pleotrichocladium	0.0038	0.600			

578


A
0.25
0.00
-0.25
NMDS2
Stress = 0.23
-0.5
0.0
0.5
B
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
NMDS2
Stress = 0.28
-0.2
0.0
0.2
NMDS1
Vegetation_Status
D
DTH
Nveg
Elevation
2492
2737
2873
2731
2759
2900

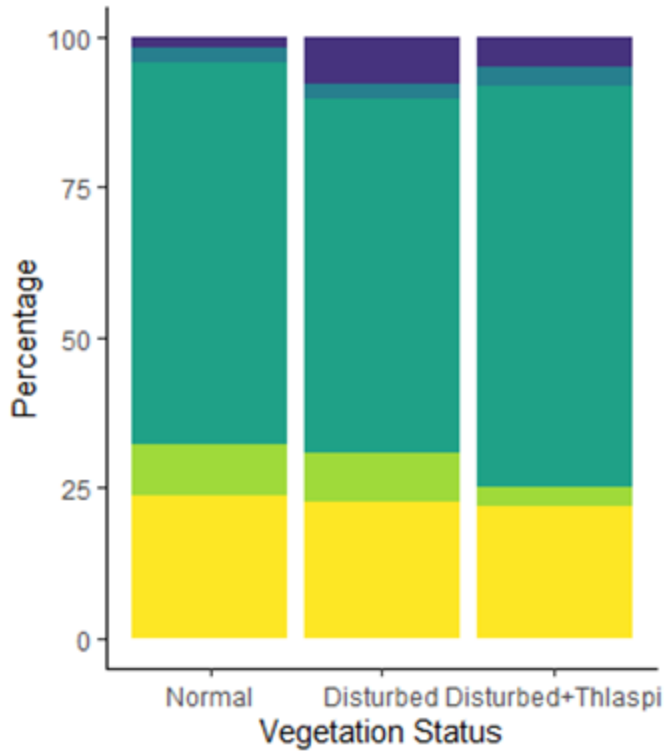
A



Classification

- Agaricomycetes
- Chytridiomycetes
- Dothideomycetes
- Eurotiomycetes
- Leotiomycetes
- Microbotryomycetes
- Mortierellomycotina
- Pezizomycetes
- Rare Fungi
- Sordariomycetes
- Tremellomycetes
- Unidentified class in phylum Ascomycota
- Unidentified phylum in kingdom Fungi

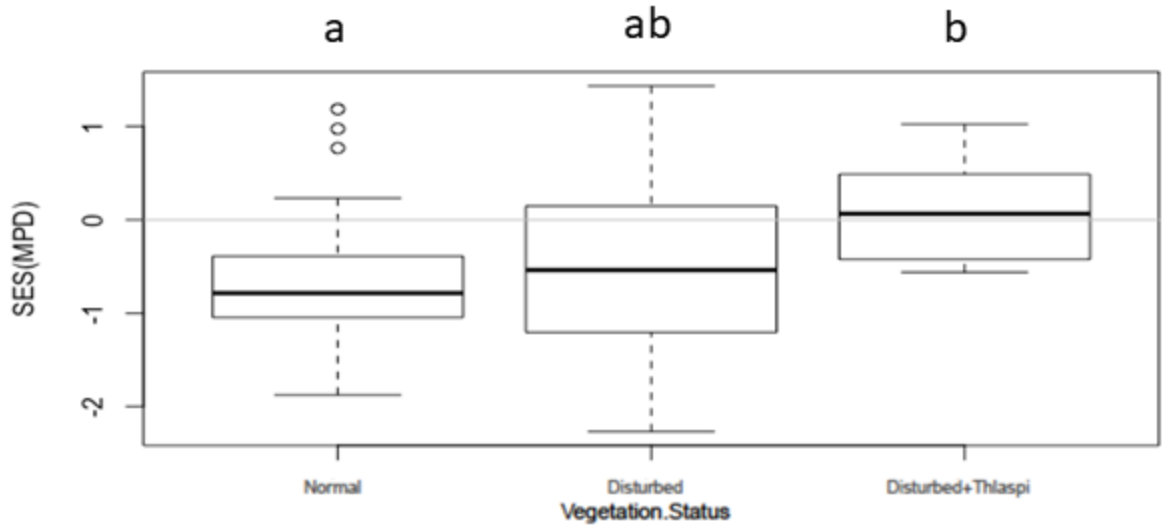
B



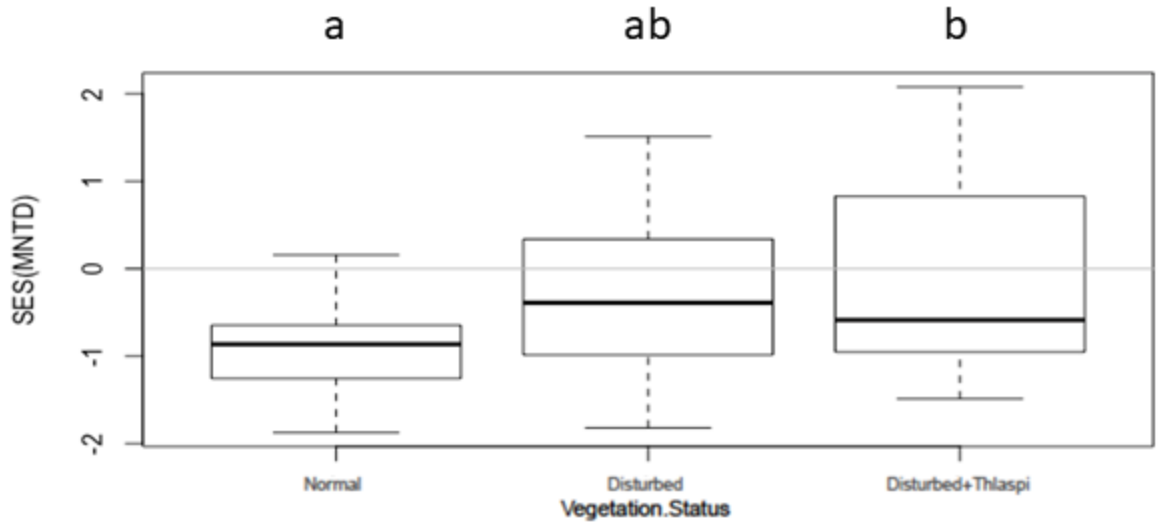
Classification

- Acaulosporaceae
- Claroideoglomeraceae
- Diversisporaceae
- Gigasporaceae
- Glomeraceae
- Unidentified family in the order Diversisporales
- Unidentified family in the order Glomerales
- Unidentified order in the class Glomeromycetes

A


a
ab
b
SES(MPD)
1
0
-1
-2
Normal
Disturbed
Disturbed+Thlaspi
Vegetation.Status

B


a
ab
b
SES(MNTD)
2
1
0
-1
-2
Normal
Disturbed
Disturbed+Thlaspi
Vegetation.Status