

The impact of elevational gradients on dark septate endophytes (DSE) and arbuscular mycorrhizal fungi (AMF) in mountains

Alex Lintner

Full Time Independent Research

Mentors: Jeremiah Henning, Jennie DeMarco, Aimée Classen

Date of Submission: August 11, 2020

Abstract

The biogeography of soil microbial communities is changing in response to increased anthropogenic disturbance, such as climate change. However, the response patterns of microbes to these rapid and accelerating changes is poorly quantified, especially when compared with our understanding of macroscopic aboveground response. The aim of this experiment was to elucidate the colonization patterns of arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) along two mountain gradients in Colorado and New Zealand. I hypothesize that, consistent with the observation that DSE are more tolerant of extreme conditions than AMF, DSE colonization is higher at high elevation, and conversely, AMF colonization is higher at low elevation. I scored stained root samples for fungal structures—the sample roots were collected from high and low elevation sites in both the Gunnison Basin, Colorado and the Tongariro National Park, New Zealand between 2014 and 2019. We found a significantly higher proportion of DSE at the high elevation site in Colorado compared to the low elevation site in Colorado. While there was a similar trend of an increasing proportion of DSE at the high elevation site in New Zealand, the difference in colonization of AMF versus DSE at high and low sites was not statistically significant. These data suggest AMF are more sensitive to environmental filters while DSE are more adaptive to abiotic stress associated with increased altitude, and they add to the growing body of work exploring the patterns and drivers of microbial biogeography.

Introduction

The impact of anthropogenic global change factors, such as climate change, on belowground microbial communities is under quantified when compared to aboveground macroscopic response (Cavicchioli et al., 2019). This oversight is easily explained—it is dark underground in the ‘black box’ of soil, and, thus, investigating the response of microbial communities has proven largely intractable until recent decades brought significant advances in DNA technologies (Fernandez and Kennedy, 2015; Tedersoo, 2017). That this hidden world of microbes, sometimes referred to as the ‘unseen majority’, is often overlooked substantially limits our understanding of how ecosystems function and are changing in response to anthropogenic disturbance (Kivlin et al., 2013). As the term ‘unseen majority’ suggests, microbes, while invisible to the naked eye, are astoundingly diverse and abundant—they constitute the foundational life support system for the earth. For instance, microorganisms are crucial for regulating carbon and nitrogen cycling (Cavicchioli et al., 2019). As global change continues to accelerate at an unprecedented scale and pace, it is urgent that we track how microbial communities are responding.

Gradients, such as mountain elevational gradients with rapid climatic shifts, are a powerful tool for monitoring species biogeography, tracking species response to global change factors, and disentangling broad-scale biodiversity mechanisms (McCain and Grytnes, 2010; Tedersoo, 2017). There is a long history of elevational studies spanning back to prominent biologists such as Linnaeus, Darwin, Wallace, and von Humboldt (Lomolino, 2001). While these scientists were focused on noticing aboveground changes in diversity with altitude, mountain gradients also help elucidate patterns of microbial biogeography and provide unique conditions for untangling the relative importance of varying abiotic and biotic factors (Kivlin et al., 2017;

Tedersoo, 2017). In addition to providing the opportunity to observe changes across elevational gradients, mountains are biodiversity hotspots with particularly high belowground biodiversity (Korner, 2004; Noroozi et al., 2018). Furthermore, mountains are especially sensitive to climate change compared to other terrestrial environments, and mountain gradients improve our understanding of threats to biodiversity from climate change (Becker and Bugmann, 2001). In sum, mountains are ideal locations to ask ecologically relevant questions about microbial biogeography and microbial response to global change factors.

Here, I explore impact of elevational gradients on the biogeography of two plant associated fungal functional groups: arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE). AMF are a thoroughly studied group of obligate plant symbionts; they are the most common and universally distributed type of mycorrhizal fungi associating with the roots of over 80 percent of vascular plants (Wang and Qiu, 2006). In addition to being pervasive, AMF-host plant symbiosis is ancient—mycorrhizal fungi were found in a fossil of an Early Devonian land plant (Remy et al., 1994) supporting the hypothesis that mycorrhizal fungi and host plant mutualism was a key development facilitating plant terrestrialization 460 to 480 million years ago (Pirozynski and Malloch, 1975). Compared to the plethora of knowledge concerning AMF, DSE are a more obscure and elusive group of likely facultative plant associated fungi (Mandyam and Jumpponen, 2005). DSE are found on over 600 plants worldwide and often co-exist with other mycorrhizal fungi such as AMF (Jumpponen and Trappe, 1998). While DSE have a broad distribution like AMF, their ecological relevance and the symbiotic status remain uncertain; DSE-plant interactions range from mutualistic to parasitic (Jumpponen, 2001). DSE are frequently found in plant roots in extreme or high stress environments such as receding glaciers (Jumpponen et al., 1998), deserts (Wu et al., 2009; Xie et al., 2017), or the artic (Newsham et al.,

2009). Their distinctive dark melanized septa and hyphae are thought to impart these fungi with greater capacity to withstand stressors at high elevation such as cold temperatures or UV exposure (Robinson, 2001).

By quantifying and analyzing observational data, the aim of this study was to explore patterns of fungal biogeography across two geographically distinct mountain clines. The purpose of this research was to untangle AMF and DSE root colonization patterns across two elevational gradients in Colorado and New Zealand. I hypothesized that consistent with the observation that DSE are more tolerant of extreme conditions than AMF, DSE colonization is higher at high elevation, and conversely, AMF colonization is higher at low elevation. Overall, I predicted there would be strong directional shifts in AMF and DSE colonization with elevational changes.

Methods

During the summers of 2014, 2015, and 2016, soil cores were collected from low (2740m; 38.71533333, -106.8226389) and high (3450m; 38.99158333, -107.0665556) elevation sites and fungal colonization was quantified across plant species in the Colorado Rocky Mountains, United States. In February 2019, Soil cores were also collected in New Zealand from low (1070m; -39.29363333333333, 175.7262944444444) and high (1610m; -39.2823972222222, 175.6208944444444) elevation sites in the Tongariro National Park. Dr. Jeremiah Henning later processed, stained, and mounted these New Zealand roots. Both the United State and New Zealand sites have a two-degree Celsius change temperature average between the high and low sites.

At the low and high elevation sites in Colorado and New Zealand, fungal colonization was measured in at least 85 percent of the plant community as determined by relative cover. A soil core was taken from beneath host plant species and the roots were extracted. Following field sample collection and root extraction, roots were cleared with ten percent potassium hydroxide (KOH) overnight in tissue cassettes (Brundrett et al., 1984; Vierheilig et al., 2005). Following clearing, the roots were acidified for one hour in two percent hydrochloric acid (HCl). After acidification, the roots were stained overnight at room temperature with a 0.01 percent w/v trypan blue solution (Koske and Gemma, 1989). Once properly stained, the roots were mounted horizontally onto glass microscope slides with polyvinyl-lacto-glycerol.

We scored the New Zealand slides for AMF and DSE fungal structures using a modified version of the magnified intersection method (Mcgonigle et al., 1990). Using a compound microscope at 40x magnification, we assessed a minimum of 50 random root intersections per slide. We determined the overall colonization of AMF and DSE as the proportion of intersections

containing AMF hyphae, arbuscules, hyphal coils, and vesicles, and DSE hyphae and microsclerotia. We also scored the presence of fungal spores, soil pathogens, and oomycetes.

We used JMP ® 14.2.0 to perform statistical analyses. We ran a Shapiro-Wilk W Test to test for normal distribution. Data distributed non-normally were log transformed. We also tested for equal variances using Levene's test. We then performed an unequal variance t-test comparing the ratio of AMF to DSE at high versus low sites in Colorado and New Zealand.

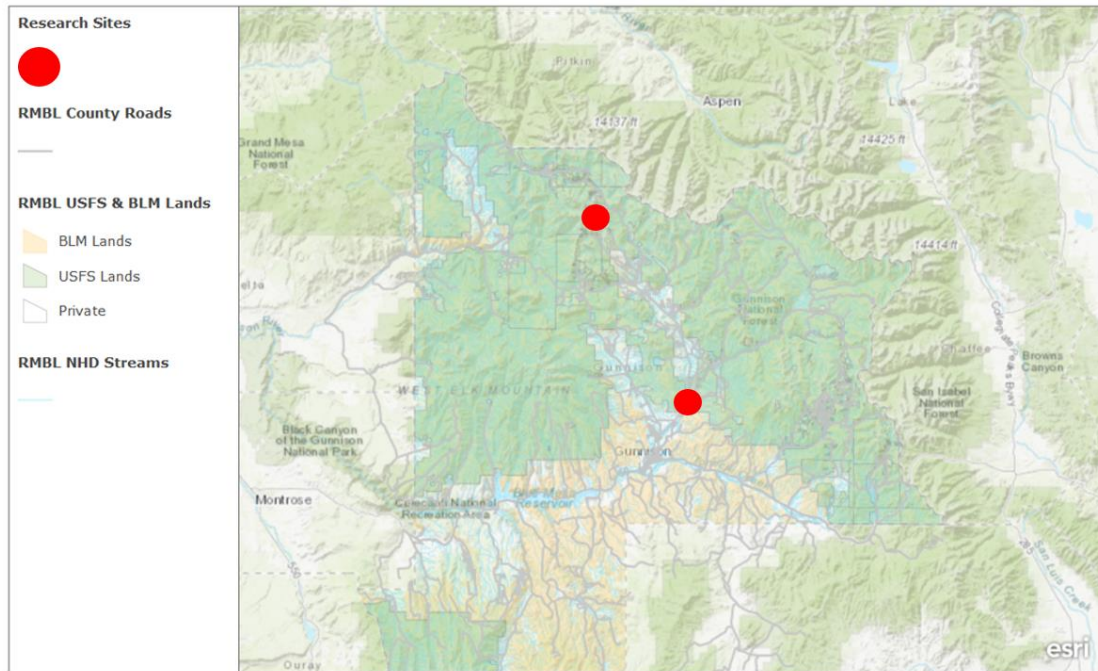


Figure 1. Map of low and high elevation sites for Colorado. Cinnamon Site (3450m; 38.99158333, -107.0665556) and Almont Site (2740m; 38.71533333, -106.8226389).

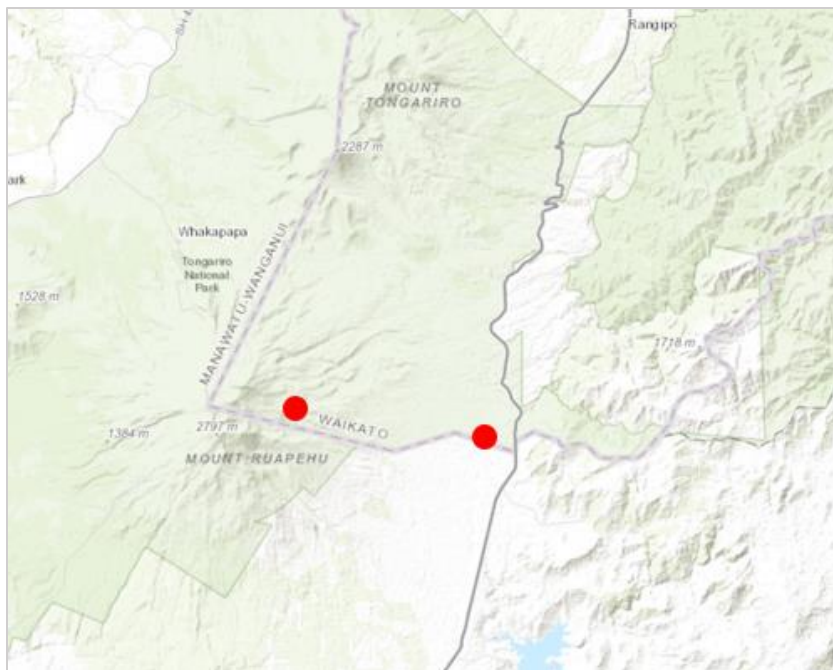


Figure 2. Map of low and high elevation sites for New Zealand. Upper Site (1610m; -39.2823972222222, 175.620894444444) and Lower Site (1070m; -39.2936333333333, 175.726294444444).

Results

All types of fungal structures scored were observed across the high and low elevation sites in both Colorado and New Zealand. All root samples except one were co-colonized with AMF and DSE, although the extent of colonization varied with elevation. There were directional shifts in the occurrence of AMF and DSE structures at high and low elevations at both sites.

For the New Zealand data, the ratios of AMF to DSE structures per slide were normally distributed and with equal variance. The Colorado ratio of AMF to DSE were not normally distributed—using a Shapiro-Wilk W Goodness-of-Fit Test we rejected the null hypotheses that these data were normally distributed ($W=0.982383$, $\text{Prob}<W$ 0.0127). We log transformed the Colorado data to correct the distribution and meet the assumption of normal distribution. The Colorado data had unequal variance—using a Levene's test we rejected the null hypothesis of equal variance (F ratio=20.6572, $df=1$, P -value<0.0001).

Both Colorado and New Zealand had a trend of a larger ratio of AMF to DSE at low elevations compared to high, suggesting increased DSE colonization or decreased AMF colonization at high elevation sites (Figure 3, Figure 4). For Colorado, we performed an unequal variance t-test and found there was a significant difference between the ratio of AMF to DSE at high and low elevations ($t=5.343$, $df=113.012$, $P<0.0001$) (Figure 3). For New Zealand, the t-test results were statistically not significant ($t=1.146$, $df=18.398$, $P=0.133$) (Figure 4).

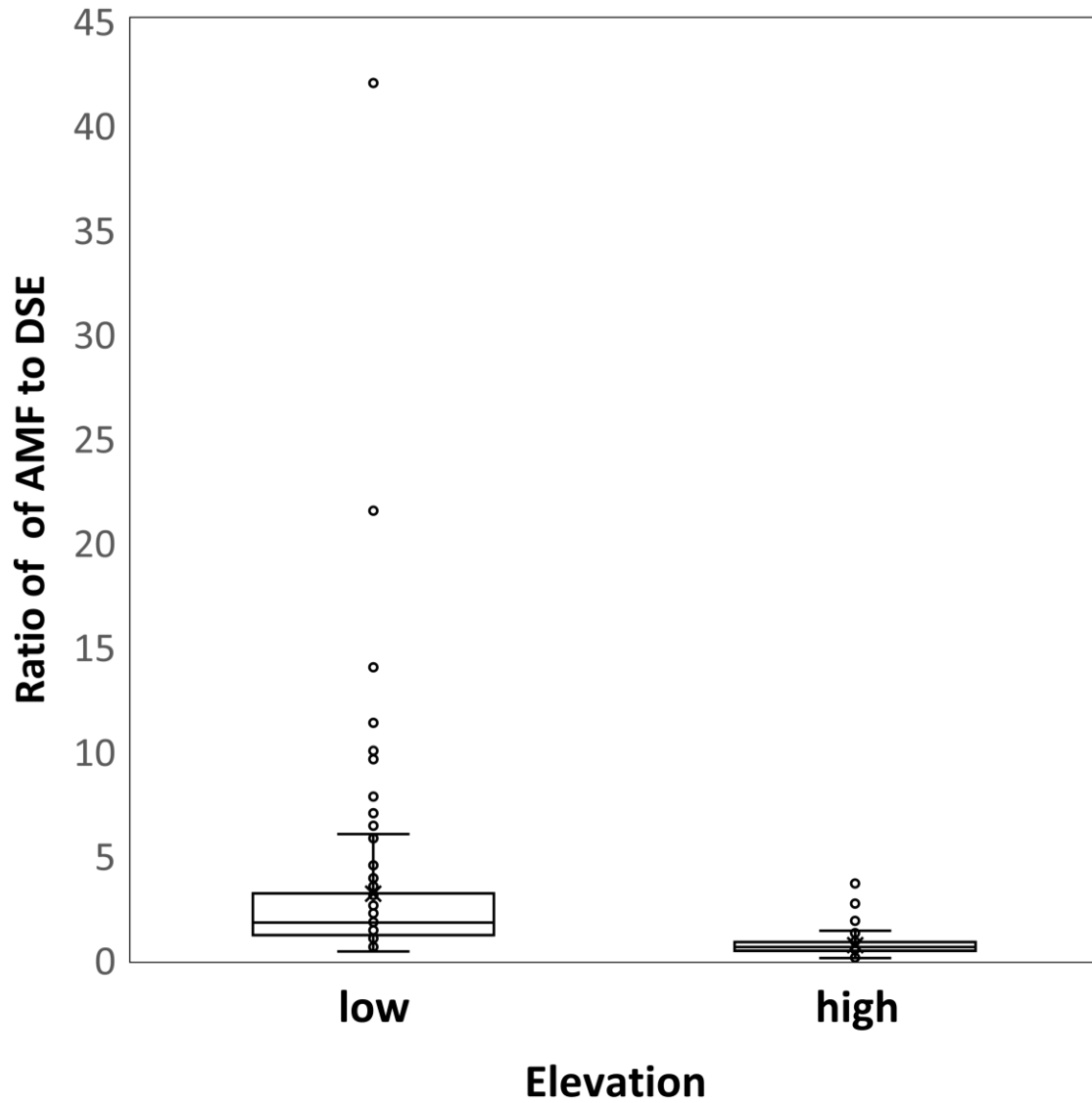


Figure 3. Ratio of the percentage of AMF to the percentage of DSE at low (2740m) and high (3460m) sites in the Rocky Mountains, Colorado. Low site (n=111) mean ratio is 3.141, and high site (n=11) mean ratio is 0.680. T-test results ($t=5.343$, $df=113.012$, $P<0.0001$).

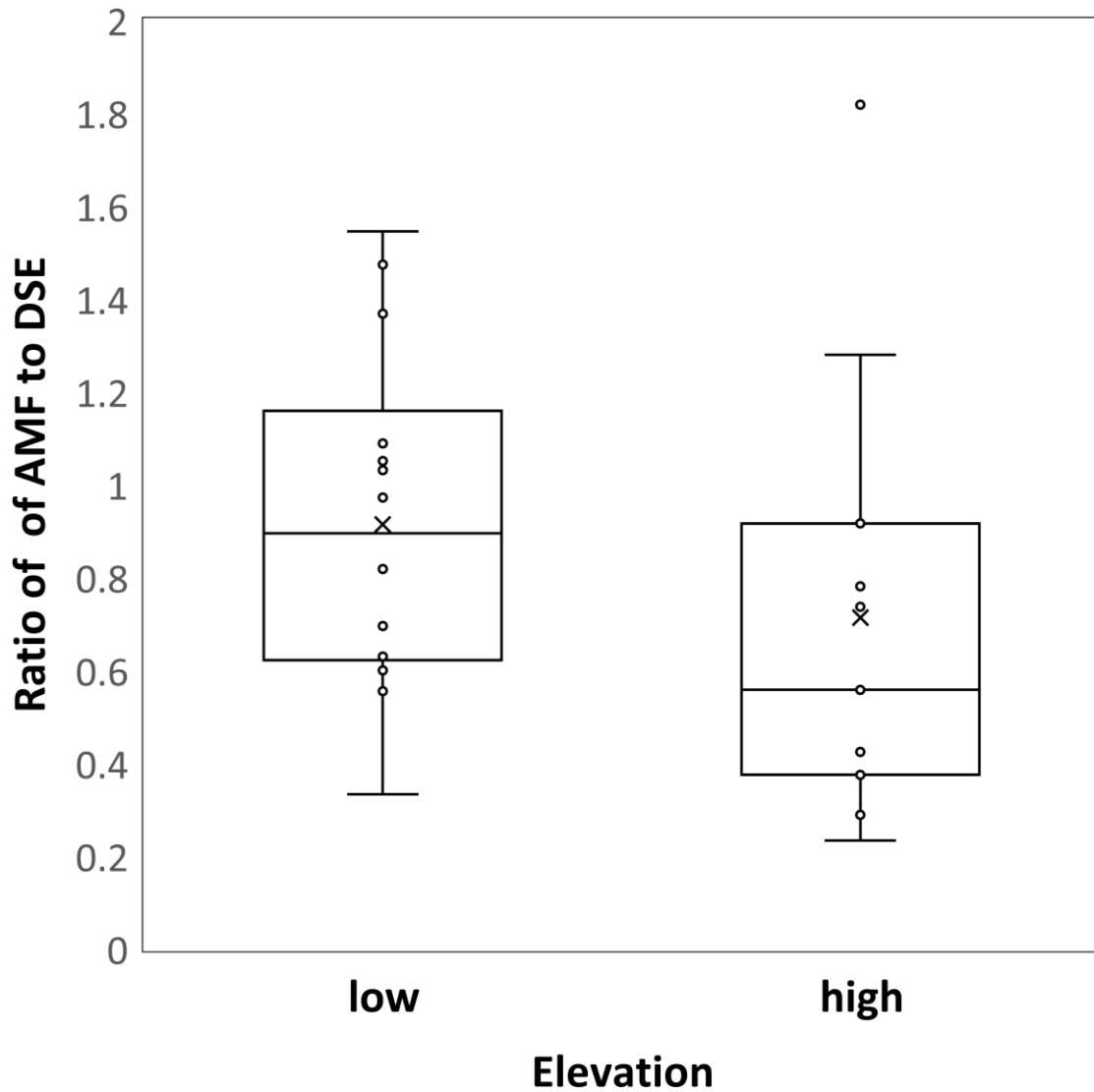


Figure 4. Ratio of the percentage of AMF to the percentage of DSE at low (1070m) and high (1610m) sites in Tongariro National Park, New Zealand. Low site (n=14) mean ratio is 0.914, and high site (n=11) mean ratio is 0.714. T-test results ($t=1.146$, $df=18.398$, $P=0.133$).

Discussion

In this study we assessed AMF and DSE colonization patterns of plant communities at high and low elevation sites in the United States and New Zealand. We were interested in understanding how these two fungal types respond to stressors along elevation gradients since anthropogenic disturbance is rapidly shifting the stressor load for microbial communities and subsequently changing the landscape distribution of AMF and DSE. Studying AMF and DSE response to stressors is also important since these fungi are in symbiotic relationships with plants and impact plant productivity, diversity, and overall ecosystem function. Furthermore, these fungi can mitigate plant abiotic stressors associated with climate change such as heat and drought (Compant et al., 2010; Kivlin et al., 2013). Studying fungal biogeography in mountains is ideal, since mountains are microbial diversity hotspots experiencing particularly rapid climate change. Across these two geographically distinct mountain ranges in this study, there was a similar trend in increasing DSE colonization relative to AMF with higher altitude. These results were consistent with our original hypothesis and in accordance with previous findings.

Previous research demonstrates that AMF and DSE respond differently to stressors in the environment. Several studies found that AMF community diversity decreases at high elevation. AMF are sensitive to environmental filtering from stressful abiotic and biotic conditions at high elevation (Egan et al., 2017). In addition to decreasing diversity, elevation also changes AMF abundance (Kotilinek et al., 2017; Lugo et al., 2018; de Mesquita et al., 2018). Above certain elevations, AMF are no longer found in plant roots (Kotilinek et al., 2017; Schmidt et al., 2008). For instance, in a study quantifying AMF and DSE colonization in plant roots along an elevational gradient (3400-6150m) in the Himalayas, AMF were not found above 5800m while their host plants ascended to 6150m, while, DSE were found across the entire gradient (Kotilinek

et al., 2017). While AMF were constrained by abiotic and biotic filters at high elevation, DSE appear better adapted to the high stress load in these locations. DSE are often recorded in roots of high elevation plants, where they partially or completely replace AMF colonization (Kotilinek et al., 2017; Schmidt et al., 2008). In extreme ecosystems where AMF are environmentally filtered, such as high elevation alpine ecosystems with slow nutrient cycling, DSE, typically considered facultative, can act as surrogate mycorrhiza, enhancing phosphorous uptake (Kotilinek et al., 2017).

Uncovering the hidden biogeography of microorganisms is a major frontier of ecological inquiry today. Similar to aboveground macroscopic communities, microbial communities are structured by a combination of interacting abiotic climatic variables (e.g. temperature, precipitation), edaphic variables (e.g. soil pH, soil moisture, soil texture, nutrient availability) and biotic factors (e.g. the host plant) (Kivlin et al., 2014; Ranelli et al., 2015). However, when contrasted with aboveground mechanisms, belowground microbial community composition is determined by a unique suite of interacting variables (Bryant et al., 2008); running against the operating assumption that microbial communities are related to plant diversity, a litany of recent scientific studies demonstrate that aboveground biodiversity is not correlated with belowground biodiversity (Fierer et al., 2011; Hendershot et al., 2017; Prober et al., 2015; Tedersoo et al., 2014). Untangling the relative importance of various abiotic and biotic factors on microbial biogeography has proved challenging, and there is an ongoing scientific debate concerning how microbial biodiversity is structured. Future studies should focus on elucidating how soil microbial communities shift with altitudinal gradients and aim to untangle the unique suite of varying abiotic and biotic factors structuring fungal biogeography. Consideration of biotic

factors, such as plant-microbe interactions, are needed to make accurate predictions about the impact of climate change on species distributions.

Literature Cited

- Becker, A., and Bugmann, H. (2001). Global Change and Mountain Regions — an IGBP Initiative for Collaborative Research. In *Global Change and Protected Areas*, G. Visconti, M. Beniston, E.D. Iannorelli, and D. Barba, eds. (Dordrecht: Springer Netherlands), pp. 3–9.
- Brundrett, M.C., Piché, Y., and Peterson, R.L. (1984). A new method for observing the morphology of vesicular–arbuscular mycorrhizae. *Can. J. Bot.* 62, 2128–2134.
- Bryant, J.A., Lamanna, C., Morlon, H., Kerkhoff, A.J., Enquist, B.J., and Green, J.L. (2008). Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. *PNAS* 105, 11505–11511.
- Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T., et al. (2019). Scientists’ warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology* 17, 569–586.
- Compant, S., Heijden, M.G.A.V.D., and Sessitsch, A. (2010). Climate change effects on beneficial plant–microorganism interactions. *FEMS Microbiology Ecology* 73, 197–214.
- Egan, C.P., Callaway, R.M., Hart, M.M., Pither, J., and Klironomos, J. (2017). Phylogenetic structure of arbuscular mycorrhizal fungal communities along an elevation gradient. *Mycorrhiza* 27, 273–282.
- Fernandez, C.W., and Kennedy, P.G. (2015). Moving beyond the black-box: fungal traits, community structure, and carbon sequestration in forest soils. *New Phytol.* 205, 1378–1380.
- Fierer, N., McCain, C.M., Meir, P., Zimmermann, M., Rapp, J.M., Silman, M.R., and Knight, R. (2011). Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* 92, 797–804.
- Hendershot, J.N., Read, Q.D., Henning, J.A., Sanders, N.J., and Classen, A.T. (2017). Consistently inconsistent drivers of microbial diversity and abundance at macroecological scales. *Ecology* 98, 1757–1763.
- Jumpponen, A. (2001). Dark septate endophytes - Are they mycorrhizal? *Mycorrhiza* 11, 207–211.
- Jumpponen, A., and Trappe, J.M. (1998). Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist* 140, 295–310.
- Jumpponen, A., Mattson, K.G., and Trappe, J.M. (1998). Mycorrhizal functioning of *Phialocephala fortinii* with *Pinus contorta* on glacier forefront soil: interactions with soil nitrogen and organic matter. *Mycorrhiza* 7, 261–265.
- Kivlin, S.N., Emery, S.M., and Rudgers, J.A. (2013). Fungal symbionts alter plant responses to global change. *American Journal of Botany* 100, 1445–1457.

- Kivlin, S.N., Winston, G.C., Goulden, M.L., and Treseder, K.K. (2014). Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. *Fungal Ecol.* 12, 14–25.
- Kivlin, S.N., Lynn, J.S., Kazenel, M.R., Beals, K.K., and Rudgers, J.A. (2017). Biogeography of plant-associated fungal symbionts in mountain ecosystems: A meta-analysis. *Diversity and Distributions* 23, 1067–1077.
- Korner, C. (2004). Mountain biodiversity, its causes and function. *Ambio* 11–17.
- Koske, R.E., and Gemma, J.N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycol. Res.*
- Kotilinek, M., Hiiesalu, I., Kosnar, J., Smilauerov, M., Smilauer, P., Altman, J., Dvorsky, M., Kopecky, M., and Dolezal, J. (2017). Fungal root symbionts of high-altitude vascular plants in the Himalayas. *Sci Rep* 7, 6562.
- Lomolino, M.V. (2001). Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography* 10, 3–13.
- Lugo, M.A., Menoyo, E., Risio Allione, L., Negritto, M.A., Henning, J.A., and Anton, A.M. (2018). Arbuscular mycorrhizas and dark septate endophytes associated with grasses from the Argentine Puna. *Mycologia* 110, 654–665.
- Mandyam, K., and Jumpponen, A. (2005). Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Stud. Mycol.* 173–189.
- McCain, C.M., and Grytnes, J.-A. (2010). Elevational Gradients in Species Richness. In *ELS*, (American Cancer Society), p.
- Mcgonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., and Swan, J.A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.*
- de Mesquita, C.P.B., Sartwell, S.A., Ordemann, E.V., Porazinska, D.L., Farrer, E.C., King, A.J., Spasojevic, M.J., Smith, J.G., Suding, K.N., and Schmidt, S.K. (2018). Patterns of root colonization by arbuscular mycorrhizal fungi and dark septate endophytes across a mostly-unvegetated, high-elevation landscape. *Fungal Ecol.* 36, 63–74.
- Newsham, K.K., Upson, R., and Read, D.J. (2009). Mycorrhizas and dark septate root endophytes in polar regions. *Fungal Ecol.* 2, 10–20.
- Noroozi, J., Talebi, A., Doostmohammadi, M., Rumpf, S.B., Linder, H.P., and Schneeweiss, G.M. (2018). Hotspots within a global biodiversity hotspot - areas of endemism are associated with high mountain ranges. *Scientific Reports* 8, 10345.
- Pirozynski, K.A., and Malloch, D.W. (1975). The origin of land plants: a matter of mycotrophism. *BioSystems* 6, 153–164.

- Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., et al. (2015). Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol. Lett.* 18, 85–95.
- Ranelli, L.B., Hendricks, W.Q., Lynn, J.S., Kivlin, S.N., and Rudgers, J.A. (2015). Biotic and abiotic predictors of fungal colonization in grasses of the Colorado Rockies. *Diversity and Distributions* 21, 962–976.
- Remy, W., Taylor, T.N., Hass, H., and Kerp, H. (1994). Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc Natl Acad Sci U S A* 91, 11841–11843.
- Robinson, C.H. (2001). Cold adaptation in Arctic and Antarctic fungi. *New Phytologist* 151, 341–353.
- Schmidt, S.K., Sobieniak-Wiseman, L.C., Kageyama, S.A., Halloy, S.R.P., and Schadt, C.W. (2008). Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. *Arct. Antarct. Alp. Res.* 40, 576–583.
- Smith, S.E., and Read, D.J. (2008). *Mycorrhizal symbiosis* (Amsterdam ; Academic Press).
- Tedersoo, L. (2017). *Biogeography of mycorrhizal symbiosis* (Cham, Switzerland : Springer,).
- Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., et al. (2014). Global diversity and geography of soil fungi. *Science* 346.
- Vierheilig, H., Schweiger, P., and Brundrett, M. (2005). An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots†. *Physiologia Plantarum* 125, 393–404.
- Wang, B., and Qiu, Y.-L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16, 299–363.
- Wu, Y., Liu, T., and He, X. (2009). Mycorrhizal and dark septate endophytic fungi under the canopies of desert plants in Mu Us Sandy Land of China. *Front. Agric. China* 3, 164–170.
- Xie, L., He, X., Wang, K., Hou, L., and Sun, Q. (2017). Spatial dynamics of dark septate endophytes in the roots and rhizospheres of *Hedysarum scoparium* in northwest China and the influence of edaphic variables. *Fungal Ecology* 26, 135–143.
- Zhao, F., Feng, X., Guo, Y., Ren, C., Wang, J., and Doughty, R. (2020). Elevation gradients affect the differences of arbuscular mycorrhizal fungi diversity between root and rhizosphere soil. *Agric. For. Meteorol.* 284, 107894.