



Evidence for a genetic basis in functional trait tradeoffs with microbial growth rate but not growth yield

Eric W. Morrison^{a,*}, Shana A. Whitney^a, Kevin M. Geyer^b, Joseph L. Sevigny^c,
A. Stuart Grandy^a, W. Kelley Thomas^c, Kristen M. DeAngelis^d, Serita D. Frey^a

^a Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH, 03824, USA

^b Department of Biology, Young Harris College, Young Harris, GA, 30582, USA

^c Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH, 03824, USA

^d Department of Microbiology, University of Massachusetts, Amherst, MA, 01003, USA

ARTICLE INFO

Keywords:

Trait tradeoffs
Functional traits
Growth rate
Carbon use efficiency
Microbial community function

ABSTRACT

Tradeoffs in microbial functional traits have been a focus of recently described ecological frameworks and of mathematical models of microbial community functioning. Tradeoffs in key traits such as growth rate, growth yield, resource acquisition, and stress tolerance may have either a genetic basis or a physiological basis, and the type of tradeoff can inform how traits are modeled and measured. Here we provide evidence that growth rate/decomposition and growth rate/stress tolerance tradeoffs have a primarily genetic basis in a phylogenetically diverse suite of ten leaf litter-inhabiting fungi. In contrast, growth yield tradeoffs with functional traits are more likely to have a physiological basis. Consideration of the type of tradeoff, genetic or physiological, should help to inform efforts to model microbial contributions to ecosystem processes, especially when considering different scales. Consideration of physiological tradeoffs may be important for understanding short-term variability (e.g., pulse events) and fine spatial scales, whereas genetic tradeoffs are likely to be useful for understanding regional- to continental-scale and medium- to long-term contributions of microbes to ecosystem processes.

Multi-trait tradeoffs in microbial functional traits have been a focus of recently described ecological frameworks (e.g., Fierer et al., 2007; Wallenstein and Hall, 2012; Wood et al., 2018; Anthony et al., 2020; Malik et al., 2020a) and of mathematical models of microbial community functioning under different environmental conditions (e.g., Moorhead and Sinsabaugh, 2006; Allison, 2012; Wieder et al., 2015). Tradeoffs in trait values are an attractive target for modeling efforts because they represent a convenient way to predict emergent community functioning under different physical conditions (Martiny et al., 2015). However, there are different kinds of tradeoffs that microbes may present that often appear to be conflated in the literature and which can inform how traits are modeled and measured.

Tradeoffs can be broadly defined as genetic versus physiological (*sensu* Stearns, 1989, 2000) for the purposes of microbial modeling efforts. Genetic tradeoffs occur when species tend to maximize one trait at the expense of another and arise through evolutionary processes (Agrawal et al., 2010). For example, different plant species have been observed to maximize either leaf photosynthetic rate or stress tolerance (i.e., the ability to maintain photosynthesis under stressful conditions),

with gains in one strategy coming at a cost to the alternative trait (Zhang et al., 2017). In contrast, physiological tradeoffs occur where a species is limited in the expression of particular traits under different conditions, primarily through energy and nutrient limitation. For example, in a highly stressful environment a species may devote more resources to production of stress resistance compounds at the expense of growth yield and/or biomass production (e.g., Malik et al., 2020b). Expression of either trait comes within the bounds encoded within the species' genome and is determined by the environment. These terms ("genetic" versus "physiological" tradeoffs) are imperfect given their variable use in the literature; however, we feel these reflect both a common understanding of the terms in evolutionary biology (e.g., Stearns 1989, 2000; Agrawal et al., 2010) and in microbial ecology (e.g., Martiny et al., 2015; Bittleston et al., 2021). Here we primarily discuss tradeoffs at the level of species as a convenient shorthand and a relevant unit for modeling efforts though we recognize that evolutionary processes may (primarily) give rise to genetic tradeoffs between individuals and populations, and similarly, physiological tradeoffs occur within individuals and populations.

* Corresponding author.. 56 College Rd., James Hall rm. 114, Durham, NH, 03824, USA.

E-mail address: eric.morrison@unh.edu (E.W. Morrison).

Genetic tradeoffs, therefore, define the range of trait values available to a community, whereas physiological tradeoffs define the traits expressed by a given community under different environmental conditions. These tradeoffs likely operate at different scales. For example, microbial community composition responds strongly to seasonal (Vořšíková et al., 2014) and decadal (DeAngelis et al., 2015) temporal variation, as well as regional (Pellissier et al., 2014) and continental (Fierer and Jackson, 2006) spatial gradients, whereas microbial communities with similar species composition may express different traits under short-term variation (e.g., according to diel cycles or pulse events; Ottesen et al., 2013). These definitions also inform how we measure potential tradeoffs. Information about genetic tradeoffs can be obtained by measuring species identity in a community given some prior information about functional potential (e.g., genomic content) or by directly measuring genomic content of a species or community. In contrast, information about physiological tradeoffs can be obtained through methods such as metatranscriptomics (i.e., measuring real-time expression of genes) or measurements of emergent traits (e.g., growth rate, growth yield) of individuals or communities under different conditions. What is yet unclear is which microbial traits, particularly those that are a common focus of modeling efforts, such as potential extracellular decomposition enzyme activity, growth rate, and growth yield, can be ascribed to species-level (i.e., genetic) versus physiological tradeoffs, and similarly, what level of variation occurs in these traits given different communities and environmental conditions.

Here we provide evidence that correlations between growth rate/decomposition potential and growth rate/stress tolerance traits have a primarily genetic basis in a phylogenetically diverse suite of ten leaf litter-inhabiting fungi. In contrast, growth yield tradeoffs with other functional traits appear to have a physiological basis. We measured growth rate and carbon use efficiency (i.e., CUE, a measure of growth yield *sensu* Malik et al., 2020a) of ten saprotrophic soil fungi under two temperatures (15 or 25 °C) and three N availability conditions (20:1, 60:1, 123:1 C:N) in liquid media using a full-factorial experimental design. Growth rate was measured by filtration-collection and weighing of dried fungal biomass at multiple time-points over a growth curve with coinciding measurements of CO₂ evolution, with CUE calculated during the exponential growth phase according to Pold et al. (2020). Sampling of the intermediate C:N media was performed by sampling of four replicate cultures at each time point, whereas for the lowest and highest C:N media three replicate growth curves were measured using separate batches of media on separate days. We therefore treat the intermediate C:N experiments as single growth estimates, and treat the three growth estimates for each of the lowest and highest C:N conditions as

independent replicates in statistical analyses. Detailed methods are available in the Supplementary Information.

Growth rate showed relatively little variation across culture conditions and instead varied primarily by species identity (Fig. 1A and B), whereas CUE displayed greater variability across the different growth conditions (Fig. 1C and D). Specifically, species identity explained 81% of the variation in growth rate (ANOVA; $R^2 = 0.816$, $P < 0.0001$) compared to 6% for growth conditions (temperature $R^2 = 0.003$, $P = 0.07$; C:N $R^2 = 0.047$, $P < 0.0001$; interaction $R^2 = 0.009$, $P = 0.01$). In contrast, species identity explained only 34% of variation in CUE ($R^2 = 0.343$, $P < 0.0001$) compared to 21% for growth conditions (temperature $R^2 = 0.172$, $P < 0.0001$; C:N $R^2 = 0.022$, $P = 0.053$; interaction $R^2 = 0.012$, $P = 0.20$; ANOVA tables are presented in Table S1).

We next used partial least squares regression (PLSR) to compare species genome content (i.e., gene counts within Gene Ontology (GO) categories; The Gene Ontology Consortium, 2019) to growth rate and CUE, using leave-one-out cross-validation (LOO-CV; Schmidtlein et al., 2012) as a measure of the predictive power of species genome content for the different growth measures. We first performed principal component analysis (PCA) to decompose growth rate and CUE into major axes of variance across growth conditions (Fig. S1) after averaging growth measures for each species within individual growth conditions to reduce residual variation and increase interpretability (i.e., treated replicates within levels of species \times growth condition as technical replicates). GO categories that were significant indicators of different PCA axes are presented in Table S2. The first PCA axis explained 96.5% of the variance in growth rate and was highly correlated with species mean growth rate ($R^2 = 0.998$, $P < 0.0001$), confirming species identity as the primary source of variation. For the CUE data, the first two PCA axes explained 52.2% and 23.7% of the variance, respectively. The first axis was highly correlated with species mean CUE ($R^2 = 0.986$, $P < 0.0001$), indicating variance related to species identity. The second axis was poorly correlated with species mean CUE ($R^2 = 0.004$, $P = 0.86$), suggesting that growth conditions drove variation on this axis.

Genome content was highly predictive of variation in growth rate between species (GR PC1, LOO-CV $R^2 = 0.795$, RMSEP = 0.037), but had poor predictive power for species differences in CUE (CUE PC1, LOO-CV $R^2 = 0.099$, RMSEP = 0.205; Fig. 2). Interestingly, genome content was a good predictor of variation in CUE across growth conditions (CUE PC2, LOO-CV $R^2 = 0.711$, RMSEP = 0.078), indicating a genetic basis for environmental variation in CUE (i.e., genome content determines physiological response range), but minimal genetic contribution to average differences in CUE between species.

Given the strong evidence of a genetic basis for species-level

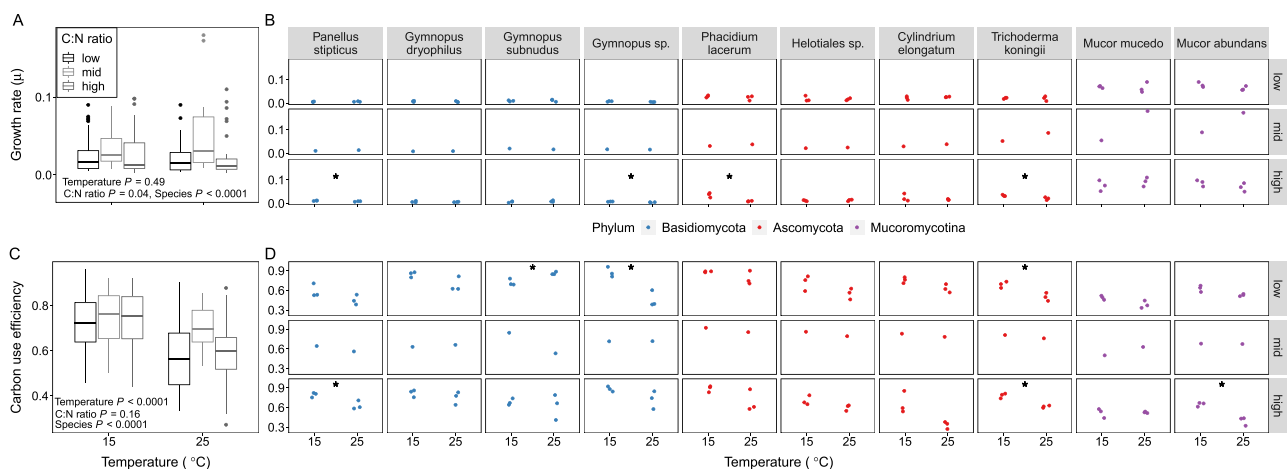


Fig. 1. Growth rate (μ) and carbon use efficiency (CUE) of ten soil fungal species measured under two temperatures and three C:N ratios in liquid growth media. Panels A and B depict growth rate averaged across species (A) or for individual species (B) under different growth conditions. Panels C and D depict CUE averages across all species (A) or for individual species (D). Asterisks in B and D indicate significant differences in respective growth measures under different temperatures.

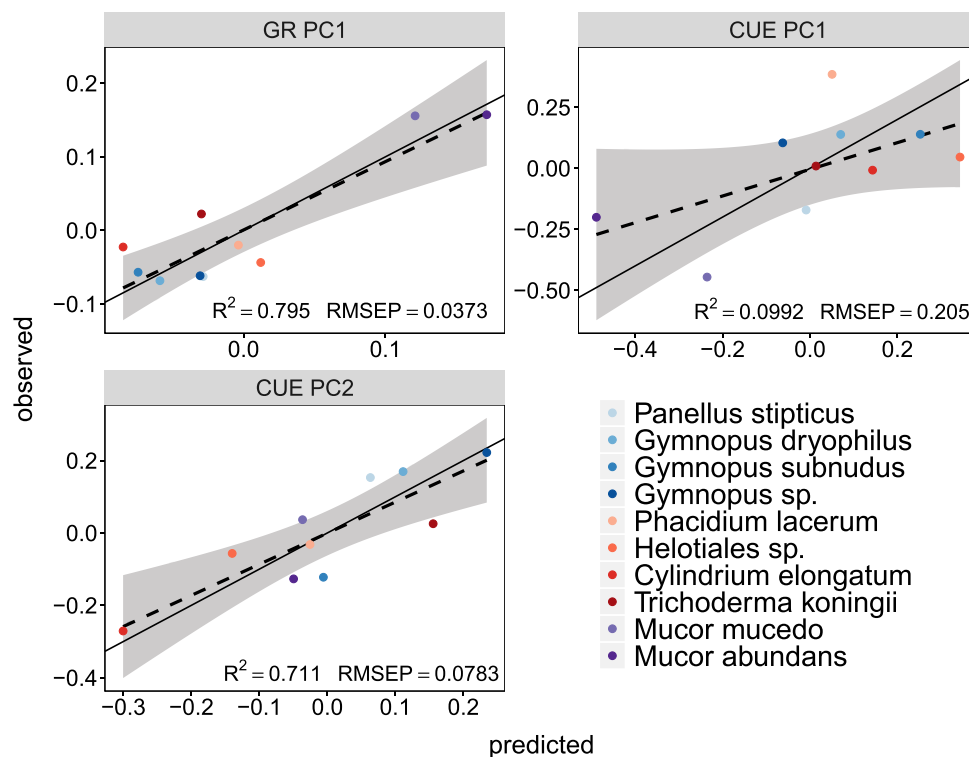


Fig. 2. Leave-one-out cross-validation (LOOCV) results from partial least squares regression (PLSR) models comparing species genome content to PCA of growth rate (GR) or carbon use efficiency (CUE). Growth rate PC1 accounted for 96.5% of variance in growth rate, and so PLSR comparisons to higher axes were not performed, whereas CUE PC1 and PC2 explained 52.2% and 23.7% of variance, respectively. The R^2 of predicted versus observed values and root mean squared error of prediction (RMSEP) are presented as indicators of predictive power.

differences in growth rate, we compared genomic growth rate indicators (as PLSR species scores) to genomic potential for decomposition and stress tolerance (*sensu* Treseder and Lennon, 2015; Supplementary Information; Fig. 3). We found a negative correlation between growth rate and decomposition potential ($r = -0.824$, $P = 0.003$), a positive correlation between growth rate and stress tolerance ($r = 0.887$, $P = 0.0006$), and a negative correlation between decomposition potential and stress tolerance ($r = -0.661$, $P = 0.038$).

Taken together our results provide some support for recently published theoretical frameworks describing microbial trait tradeoffs. For example, we find support for a genetic tradeoff between resource acquisition and stress tolerance as proposed in the recently described Y-A-S framework (Malik et al., 2020a), whereby species with higher decomposition capacity have lower capacity to tolerate stress. However, we also show that growth rate displayed a positive association with stress tolerance, suggesting that microbes that are adapted to high-stress conditions may also display high growth rates under favorable conditions. Rather than a growth rate/stress tolerance genetic tradeoff, this pattern is more consistent with an “exploit-and-wait” or “growth-and-dormancy” strategy, wherein a species exploits available resources under favorable conditions and lies dormant under suboptimal conditions (e.g., Lennon and Jones, 2011). Importantly, we find that growth rate is conserved at the species level, and we demonstrate evidence of a genetic basis for these differences, whereas we find lesser evidence for species-level conservation of growth yield (i.e., CUE) in the fungi examined. Instead, growth yield is an apparently highly plastic trait, wherein genomic content may contribute to the range of growth yield expressed. While our analyses are restricted to soil fungi, phylogenetically diverse bacteria also demonstrate high plasticity in CUE and little evidence for genomic underpinnings (Pold et al., 2020, but see Saifuddin et al., 2019), suggesting our results are generalizable across broad microbial groups.

We therefore suggest that consideration of the type of tradeoff, genetic or physiological, can help to inform efforts to model microbial contributions to ecosystem processes. Either type of tradeoff may be more informative at different scales, and different metrics should be

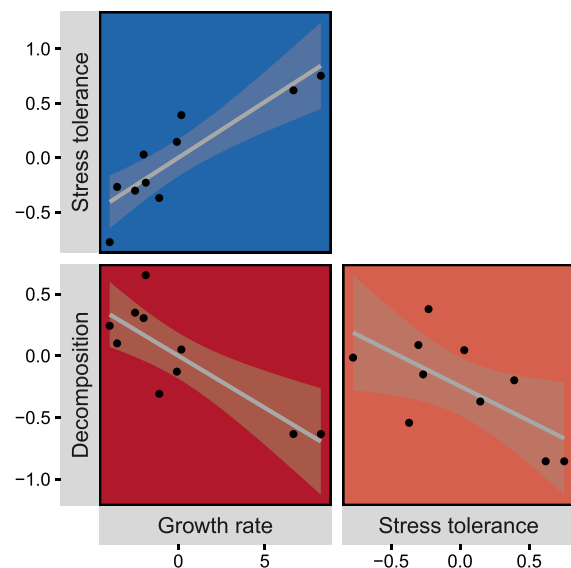


Fig. 3. Pairwise comparisons of growth rate (as predicted species scores from PLSR models) to genomic measures of decomposition and stress tolerance potential (*sensu* Treseder and Lennon, 2015; see Supplementary Information for details). Decomposition and stress tolerance were calculated as genomic investment by summing counts in each gene family category and dividing by genome size (i.e., the percentage of genes per genome in each family) and then performing PCA to determine the primary major axes of variation in counts across different gene families (e.g., lignin versus cellulose decomposition). Box color represents the strength of the correlation with red indicating negative and blue indicating a positive correlation, respectively, and darker values indicating stronger correlations. Points indicate individual species. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

used as indicators of each type of tradeoff. For example, transcriptomic metrics of CUE are under development (Hasby et al., 2021) that could help describe physiological tradeoffs, and it is likely that yield/acquisition and yield/stress tolerance tradeoffs (*sensu* Malik et al., 2020a) operate on a physiological basis (e.g., Anthony et al., 2020; Malik et al., 2020b). Classification of microbial genetic tradeoffs should prove useful for modeling regional- to continental-scale and medium- to long-term contributions of microbes to ecosystem processes, particularly C cycling, and for further development of ecological models of microbial adaptive strategies or life-history traits.

Accession numbers

Sequence data are available in NCBI SRA under accession number PRJNA551418.

Declaration of competing interest

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

Acknowledgements

This work was funded in part by the New Hampshire Agricultural Experiment Station. This is Scientific Contribution Number 2815. This work was supported by the U.S. Department of Energy Genomic Science Program (DE-S0016590).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2022.108765>.

References

- Agrawal, A., Conner, J., Rasmann, S., 2010. Tradeoffs and negative correlations in evolutionary ecology. In: Bell, M.A. (Ed.), *Evolution after Darwin: the First 150 Years*. Sinauer Associates, Sunderland, Massachusetts, pp. 243–268.
- Allison, S., 2012. A trait-based approach for modelling microbial litter decomposition. *Ecology Letters* 15, 1058–1070.
- Anthony, M.A., Crowther, T.W., Maynard, D.S., van den Hoogen, J., Averill, C., 2020. Distinct assembly processes and microbial communities constrain soil organic carbon formation. *One Earth* 2, 349–360. <https://doi.org/10.1016/j.oneear.2020.03.006>.
- Bittleston, L.S., Freedman, Z.B., Bernardin, J.S., Grothjan, J.J., Young, E.B., Record, S., Braiser, B., Gray, S.M., 2021. Exploring microbiome functional dynamics through space and time with trait-based theory. *mSystems* 6 (4), e0053021. <https://doi.org/10.1128/mSystems.00530-21>.
- DeAngelis, K.M., Pold, G., Topçuoğlu, B.D., van Diepen, L.T.A., Varney, R.M., Blanchard, J.L., Melillo, J., Frey, S.D., 2015. Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology* 6, 104. <https://doi.org/10.3389/fmicb.2015.00104>.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* 103, 626–631.
- Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. <https://doi.org/10.1890/05-1839>.
- Hasby, F.A., Barbi, F., Manzoni, S., Lindahl, B.D., 2021. Transcriptomic markers of fungal growth, respiration and carbon-use efficiency. *FEMS Microbiology Letters* 368. <https://doi.org/10.1093/femsle/fnab100>.
- Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology* 9, 119–130. <https://doi.org/10.1038/nrmicro2504>.
- Malik, A.A., Martiny, J.B.H., Brodie, E.L., Martiny, A.C., Treseder, K.K., Allison, S.D., 2020a. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal* 14, 1–9. <https://doi.org/10.1038/s41396-019-0510-0>.
- Malik, A.A., Swenson, T., Weihe, C., Morrison, E.W., Martiny, J.B.H., Brodie, E.L., Northen, T.R., Allison, S.D., 2020b. Drought and plant litter chemistry alter microbial gene expression and metabolite production. *The ISME Journal* 14, 2236–2247. <https://doi.org/10.1038/s41396-020-0683-6>.
- Martiny, J.B.H., Jones, S.E., Lennon, J.T., Martiny, A.C., 2015. Microbiomes in light of traits: a phylogenetic perspective. *Science* 350, aac9323–aac9323-8. <https://doi.org/10.1126/science.aac9323>.
- Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. *Ecological Monographs* 76, 151–174. [https://doi.org/10.1890/0012-9615\(2006\)076\[0151:ATMOLD\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2).
- Ottesen, E.A., Young, C.R., Eppley, J.M., Ryan, J.P., Chavez, F.P., Scholin, C.A., DeLong, E.F., 2013. Pattern and synchrony of gene expression among sympatric marine microbial populations. *Proceedings of the National Academy of Sciences* 110, E488–E497. <https://doi.org/10.1073/pnas.1222099110>.
- Pellissier, L., Niculita-Hirzel, H., Dubuis, A., Pagni, M., Guex, N., Ndiribe, C., Salamin, N., Xenarios, I., Goudet, J., Sanders, I.R., Guisan, A., 2014. Soil fungal communities of grasslands are environmentally structured at a regional scale in the Alps. *Molecular Ecology* 23, 4274–4290. <https://doi.org/10.1111/mec.12854>.
- Pold, G., Domeignoz-Horta, L.A., Morrison, E.W., Frey, S.D., Sistla, S.A., DeAngelis, K.M., 2020. Carbon use efficiency and its temperature sensitivity covary in soil bacteria. *mBio* 11, e02293. <https://doi.org/10.1128/mBio.02293-19>.
- Saifuddin, M., Bhatnagar, J.M., Segre, D., Finzi, A.C., 2019. Microbial carbon use efficiency predicted from genome-scale metabolic models. *Nature Communications* 10, 3568. <https://doi.org/10.1038/s41467-019-11488-z>.
- Schmidtlein, S., Feilhauer, H., Bruehlheide, H., 2012. Mapping plant strategy types using remote sensing. *Journal of Vegetation Science* 23, 395–405. <https://doi.org/10.1111/j.1654-1103.2011.01370.x>.
- Stearns, S.C., 1989. Trade-offs in life-history evolution. *Functional Ecology* 3, 259–268. <https://doi.org/10.2307/2389364>.
- Stearns, S.C., 2000. Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* 87, 476–486. <https://doi.org/10.1007/s001140050763>.
- The Gene Ontology Consortium, 2019. The gene Ontology resource: 20 years and still GOing strong. *Nucleic Acids Research* 47, D330–D338. <https://doi.org/10.1093/nar/gky1055>.
- Treseder, K.K., Lennon, J.T., 2015. Fungal traits that drive ecosystem dynamics on land. *Microbiology and Molecular Biology Reviews: Microbiology and Molecular Biology Reviews* 79, 243–262. <https://doi.org/10.1128/MMBR.00001-15>.
- Voríšková, J., Brabcová, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytologist* 201, 269–278. <https://doi.org/10.1111/nph.12481>.
- Wallenstein, M.D., Hall, E.K., 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry* 109, 35–47. <https://doi.org/10.1007/s10533-011-9641-8>.
- Wieder, W.R., Grandy, A.S., Kallenbach, C.M., Taylor, P.G., Bonan, G.B., 2015. Representing life in the Earth system with soil microbial functional traits in the MIMICS model. *Geoscientific Model Development* 8, 1789–1808. <https://doi.org/10.5194/gmd-8-1789-2015>.
- Wood, J.L., Tang, C., Franks, A.E., 2018. Competitive traits are more important than stress-tolerance traits in a cadmium-contaminated rhizosphere: a role for trait theory in microbial ecology. *Frontiers in Microbiology* 9, 121. <https://doi.org/10.3389/fmicb.2018.00121>.
- Zhang, Y.-J., Sack, L., Cao, K.-F., Wei, X.-M., Li, N., 2017. Speed versus endurance tradeoff in plants: leaves with higher photosynthetic rates show stronger seasonal declines. *Scientific Reports* 7, 42085. <https://doi.org/10.1038/srep42085>.