



Excluding arbuscular mycorrhiza lowers variability in soil respiration but slows down recovery from perturbations

STAVROS D. VERESOGLOU ^{1,†} GAOwen YANG ^{1,2} MAGKDI MOLA,¹ ANNETTE MANNTSCHKE,¹ MORITZ MATING,¹ MANFRED FORSTREUTER,¹ AND MATTHIAS C. RILLIG ^{1,2}

¹Institut für Biologie, Freie Universität Berlin, Berlin, Germany

²Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin D-14195 Germany

Citation: Veresoglou, S. D., G. Yang, M. Mola, A. Manntsche, M. Mating, M. Forstreuter, and M. C. Rillig. 2020. Excluding arbuscular mycorrhiza lowers variability in soil respiration but slows down recovery from perturbations. *Ecosphere* 11(12):e03308. 10.1002/ecs2.3308

Abstract. The role of mutualisms in mediating temporal stability in an ecosystem has been debated extensively. Here, we focus on how a ubiquitous mutualism, arbuscular mycorrhiza, influences temporal stability of a key ecosystem process, ecosystem respiration. We discriminated between two forms of temporal stability, temporal variability and resilience, and hypothesized that excluding arbuscular mycorrhiza would be detrimental for both of them. We analyzed a set of 10 parallel manipulation experiments to assess how excluding arbuscular mycorrhiza modulates temporal stability compared to other common experimental factors. We quantified the temporal variability of ecosystem respiration and the resilience to experimental perturbations (i.e., pulses, stresses, and a disturbance) following manipulations of mycorrhizal state. We observed lower temporal variability in the absence of arbuscular mycorrhiza in discord to our main hypothesis. Manipulating arbuscular mycorrhiza had a stronger impact on temporal variability than the pulse (application of urea), the stress (addition of salt), and a disturbance (experimental defoliation) but weaker than excluding primary producers or comparing across different plant species. Resilience to experimental perturbations declined in non-mycorrhizal microcosms. We present an empirical study on how mutualisms impact temporal stability. Arbuscular mycorrhiza differentially alters temporal variability and resilience, highlighting that generalizing across different forms of temporal stability could be misleading.

Key words: arbuscular mycorrhiza; experimental perturbations; Glomeromycotina; resilience; resistance; respiration; temporal stability; transition thresholds.

Received 7 July 2020; accepted 14 July 2020; final version received 2 October 2020. Corresponding Editor: Debra P. C. Peters.

Copyright: © 2020 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† E-mail: sveresoglou@zedat.fu-berlin.de

INTRODUCTION

Ecological processes are subject to continuous environmental change and as a result vary in time within an ecosystem (Halley 1996, Denny et al. 2004). Some ecosystems are less responsive to environmental change than others. We describe the property of an ecosystem to persist and absorb change and disturbance resulting from environmental variability as temporal stability (Holling 1973, Donohue et al. 2016).

Temporal stability can confer sustainability to ecosystems (Loreau and de Mazancourt 2013) and secure a constant delivery of associated ecosystem services (Donohue et al. 2016).

Temporal stability can take various forms, the most important of which are the temporal variability, depicting the variance of an ecosystem process over time; the resistance, describing the property of an ecosystem parameter to stay unchanged over time; and the resilience, describing the rate at which a system returns to

equilibrium after a perturbation (Pimm 1984, Hodgson et al. 2015). Several authors have voiced concerns that the existing literature falls short of addressing which properties of an ecosystem confer temporal stability (Thébaud and Fontaine 2010, Eisenhauer et al. 2011, O'Connor and Donohue 2013). An additional limitation is that the bulk of the literature addressing temporal stability focuses on a single component of stability which is typically the temporal variability of plant biomass (Donohue et al. 2016). Because many of these terms are used inconsistently in the literature (Hodgson et al. 2015, Donohue et al. 2016), it is desirable to define them clearly here. For the purposes of this article, we adapt the definitions from Hodgson et al. (2015), describing temporal variability as the coefficient of variation of an ecosystem process, resistance as the ability of a system to resist

change over time despite recurrent environmental change and resilience the return time to an equilibrium following a destabilizing perturbation (Fig. 1a).

There are two main ways to infer temporal stability in an ecosystem. The first way, to which we refer as stability analysis (i.e., which represents the form we describe in this article as structural stability), uses stability criteria of the underlying representations of the interactions that occur between species (i.e., their ecological interaction networks, May 1972, Allesina and Tang 2012). The stability analysis approach was developed by May (1972) and yields a theoretical probability that an ecological interaction network will persist through environmental perturbations and thus expresses structural stability. Alternatively, it is possible to assess the functional temporal stability of an ecosystem by comparing ecosystem process

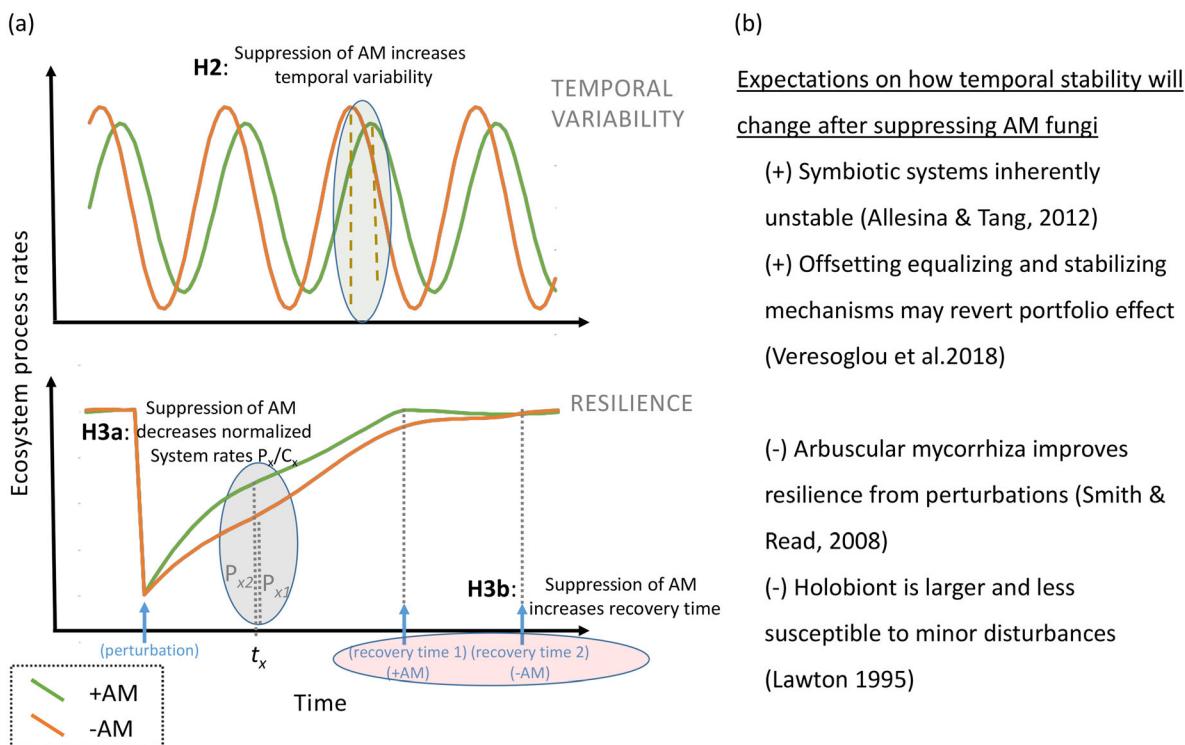


Fig. 1. (a) Overview of the two hypotheses addressing how excluding arbuscular mycorrhiza alters temporal stability. Hypothesis 2 (H_2) postulates that excluding AM would increase the temporal variability in the ecosystem; hypothesis 3 proposes that excluding arbuscular mycorrhiza would decrease the metrics of normalized system state (H_{3a}) and prolong recovery time (H_{3b}). (b) We propose a set of four key mechanisms through which excluding arbuscular mycorrhiza could alter ecosystem stability. These are discussed in detail in the main manuscript.

rates over time or in space (Tolkkinen et al. 2015, Guelzow et al. 2017). The most widely used tool to assess functional temporal stability (i.e., which represents the form we describe in this article as temporal stability) is via computing the coefficient of variation of an ecosystem process in time (i.e., temporal variability; Pimm 1984). Much of the literature assessing how stability scales with diversity has used this approach (Tilman and Downing 1994, de Mazancourt et al. 2013, Loreau and de Mazancourt 2013). Expectations on structural stability (Butterfield 2009, Allesina and Tang 2012) can occasionally be incongruent with those from temporal stability approaches (Pachepsky et al. 2002, Loreau and de Mazancourt 2013, Yang et al. 2014). As an example, structural stability flags complex food webs as inherently unstable (May 1972), which is at a mismatch with empirical data on how diversity scales with temporal stability (Tilman and Downing 1994).

A controversial topic of particular interest is how mutualisms alter structural and temporal stability, with some studies reporting increases (Pachepsky et al. 2002) and others decreases (Allesina and Tang 2012). Arbuscular mycorrhizal (AM) associations represent ubiquitous trophic mutualisms between the roots of terrestrial plants and fungi in the Glomeromycotina. The mutualism typically enhances ecosystem processes such as plant biomass, litter decomposition, and soil aggregation (Smith and Read 2008) but also intensifies interspecific plant competition (Veresoglou et al. 2018a) and could thus alter ecosystem stability via diverse mechanisms (Fig. 1b). Relatively few studies exist on how the AM symbiosis influences temporal stability (Wurzburger et al. 2017). By contrast, there have been several studies addressing how groups of soil biota other than AM influence temporal stability (de Vries et al. 2012, Griffiths and Philippot 2013, Pellkofer et al. 2016, Roger et al. 2016, Delgado-Baquerizo et al. 2017, Yang et al. 2018). A reason could be that the influence of AM associations on temporal stability is small and of little ecological importance. Arbuscular mycorrhiza is expected to reduce the structural stability of an ecosystem if mutualistic interactions destabilize structural stability as shown in Allesina and Tang (2012) or if because of their low host specificity they increase the complexity (i.e., the average number of links per species in an ecological

network) and connectance (i.e., the proportion of possible links between species that are realized in an ecological network) of the resulting ecological interaction networks (May 1972, Allesina and Tang 2012). At the same time, AM associations can promote ecosystem rates for a range of processes (e.g., plant biomass production, van der Heijden et al. 1998; soil aggregation, Leifheit et al. 2014). If higher rates of ecosystem processes foster higher temporal stability as predicted by Yachi and Loreau (1999), ecosystems with AM could also be more temporally stable. Yang et al. (2014), for example, showed that adding the fungicide benomyl consistently increased the temporal coefficient of variation of plant biomass in an experimental grassland, suggesting that the combined suppression of the AM and general fungal community and the concurrent increased availability of nutrients as a result of benomyl application (i.e., mineralization of fungal biomass and addition of an N-rich compound) decrease temporal stability. It is also unclear whether AM influences temporal variability, resistance, and resilience of an ecosystem in a comparable way because few studies make such a distinction.

To address this knowledge gap, we here analyzed temporal data on ecosystem respiration, to estimate the rate at which carbon (C) was cycling in our model ecosystems. We did so in a series of 10 parallel glasshouse microcosm experiments in which AM state represented an experimental manipulation out of a pool of six experimental manipulations. These experiments address mechanisms underpinning AM-induced modifications in temporal stability. Our objectives were to obtain estimates of relative importance of arbuscular mycorrhiza on temporal stability and determine the effects of mycorrhizal state on different types of temporal stability. We differentiated here between temporal variability and resilience. We hypothesized that the arbuscular mycorrhiza under our specific environmental and experimental temporal settings is of lower importance for the temporal stability of ecosystem respiration than other experimental perturbations such as fertilization events, plant defoliation, and changes in salinity (based on the premise that there is so little research on them; hypothesis 1). We further hypothesized that, in agreement with Yang et al. (2014), we would observe in the

microcosms without AM fungi a higher temporal variability (hypothesis two) and a lower resilience to experimental perturbations (hypothesis three; Fig. 1).

MATERIALS AND METHODS

Design considerations

Large multifactorial experiments have high recurrence rates of false positives (i.e., Type I statistical errors) or contain interaction terms which pose difficulties to interpret (Smith et al. 2002, Veresoglou 2015). We used here, instead, a set of parallel bifactorial experiments with three replicates, each testing two of the six experimental factors (plant species, presence of plants, arbuscular mycorrhiza, addition of urea (representing a pulse), addition of salt (NaCl; representing a stress) and defoliation (representing a disturbance); each parallel experiment consisted of 12 microcosms, for a total of 120 microcosms; Table 1). The experimental settings of individual experiments are presented in detail in Appendix S1. The rationale of our experimental design was to yield independent (i.e., there were 10 independent experiments) estimates (the experimental factors, with the exception of defoliation, were replicated across the 10 experimental designs between two and seven times) of relative importance for each factor which were replicated across experiments. Given that the degrees of freedom were identical for all experimental factors (i.e., there was one degree of freedom in the

nominator and eight degrees of freedom in the denominator when calculating the *F* statistics), we could infer on their relative ecological importance by comparing respective sums of squares or *R*² values following variance partitioning (here, we used *R*² values; analysis Dp1 in Appendix S2). In Table 1, we further rationalize the way we sampled parameter space so that we focus on AM but simultaneously address the other experimental factors.

Experimental design

In setting up the experiments, we used as experimental units 0.5 L conical microcosms (20 cm height; 5.5 cm maximal diameter) filled with an 1:1 mixture of sand and an Albic Luvisol containing 1.87% total C, 0.12% total N and a pH (CaCl₂) of 5.9 (Rillig et al. 2010). The soil had been collected approximately a month prior to the experiment, air dried, sieved through a 5 mm sieve, and got steam-sterilized. Then, it received depending on AM treatment per inoculated microcosm around 1000 spores of *Rhizophagus irregularis* (SYMPLANTA-001, SYMPLANTA, Darmstadt, Germany) approximately 1 cm below the soil surface. In the planted microcosms, depending on treatment, we added three seeds of either *Plantago lanceolata*, *Prunella vulgaris*, or did not add seeds. The specific plant species were chosen because they represent two AM-associating forbs with a wide distribution and which commonly co-occur in natural habitats (Veresoglou et al. 2018a). Seeds were bought from Appels

Table 1. List of the experimental factors across the 10 bifactorial experiments (represented here by unique experimental IDs as columns).

Factor	1	2	3	4	5	6	7	8	9	10
Plant species (<i>Plantago</i> vs. <i>Prunella</i>)				x		x				
Primary producers (present vs. absent)	x	x	x							x
Mycorrhiza (yes vs. no)				x	x	x	x	x	x	x
Addition of urea (yes vs. no)		x	x						x	x
Addition of NaCl (yes vs. no)	x				x		x			
Defoliation (yes vs. no)								x		

Notes: Each experiment was fully factorial with three replicates. The 10 experiments differed in relation to their experimental settings (rows), and they are presented in detail in Appendix S1. We highlight with “x” symbols instances where in an experiment we included an experimental setting as a manipulation treatment. The design of the experiments aimed at addressing the ecological importance of mycorrhiza (which for this reason is represented in seven of the experiments) over other manipulations. Mycorrhiza cannot establish in the absence of compatible hosts, and thus, the manipulations plants and mycorrhiza were incompatible; we addressed manipulations of primary producers describing whether a plant had been sown in the pots or not in three experiments. We aimed to replicate each of the other factors (i.e., Plant Species having the levels *Plantago lanceolata* and *Prunella vulgaris*; addition of urea having the levels yes or no; addition of NaCl having the levels yes or no; and defoliation having the levels yes or no) in combination to mycorrhiza (i.e., addition of spores of *Rhizophagus irregularis* or not) in two experiments. We limited the pool of factors that we combined with plants into two: addition of urea and addition of NaCl.

Wilde Samen, Darmstadt, Germany; in our experiment, germination rates exceeded 90% which meant, however, that a few planted microcosms contained at harvest only a single plant; and all planted microcosms contained at harvest a minimum of one actively growing plant. Two weeks after sowing (i.e., to minimize losses of seeds from fungal infections), we added 10 mL of a microbial wash (1:10 mix of freshly collected unsterilized Albic Luvisol which we describe earlier and water stirred for 30 min and sieved through a 20 µm sieve) per microcosm. Thirty-seven days after sowing, a time span sufficient for the establishment of AM fungi (Jansa et al. 2008), we imposed a series of abrupt changes (i.e., experimental perturbations).

Experimental perturbations comprised, depending on treatment and experiment, a fertilization pulse (i.e., addition of urea at a rate of 100 mg/kg of soil), a stress (i.e., addition of NaCl at a rate of 0.5 g/kg), or a disturbance (i.e., experimental defoliation, where we removed approximately half of the leaves from the plants in the microcosms). The specific rates of application of urea and NaCl and defoliation are common in AM studies (Chaudhary et al. 2016) and mimic anthropogenic activities in light of ongoing land use intensification (Pouzols et al. 2014). In the rest of the manuscript, we refer to this set of manipulations as perturbators. We varied the number of perturbators per experiment between zero and one. The growth settings throughout the controlled experiments were as follows: day temperature 20°C; night temperature 18°C; day duration from 06:00 till 19:30; and no supplemented lighting (i.e., the experiment was carried out from the end of May till the beginning of July). Watering was carried out daily.

Measurements

Two days after imposing the perturbators (i.e., 37 d after sowing) and over a period of ten days, we assayed each microcosm for ecosystem respiration on four different days (Appendix S1). We define here ecosystem respiration as the aggregate of soil and dark (i.e., plant respiration in the absence of photosynthesis) respiration which is indicative of the speed with which C is cycling in the ecosystem. We carried out the measurements in the dark to stop photosynthesis in customized cylindrical chambers (30 cm height and 5.5 cm diameter). Because we had capacity for 60

microcosms per day, we blocked experiments into two main blocks. Experiments belonging to the same block were assayed for ecosystem respiration on the same days but in random order (Appendix S1).

After the last assay of ecosystem respiration in the earlier mentioned four non-destructive harvests, we additionally assayed (i.e., on the same day we assayed ecosystem respiration; both blocks together) total dry aboveground biomass to serve as a proxy of plant biomass. Aboveground biomass was cut at ground level and was dried to constant weight at 60°C. Representative pieces of roots were stained in ink and vinegar (Vierheiling et al. 1998). To verify that root colonization with AM fungi occurred, visualization of root fragments at 200× magnification was carried out, revealing successful and comparable to earlier studies with the specific combinations of soil and plant (Rillig et al. 2010, Siddiky et al. 2012, Veresoglou et al. 2019); however, because AM fungi were only used as a treatment, we did not quantify root colonization.

Statistical analyses

Our statistical analysis had the following three objectives: Assess relative ecological importance of excluding arbuscular mycorrhiza in relation to the other experimental manipulations (i.e., pulse-urea, stress-NaCl and disturbance-defoliation; hypothesis 1); evaluate how excluding AM alters temporal variability (hypothesis 2); and infer how excluding AM changes the resilience of the ecosystems (i.e., microcosms) to the perturbators we used (hypothesis 3).

To address hypothesis 1, we fitted two-way ANOVAs separately to each individual bifactorial experiment with the two experimental factors as predictors. The response variable was the coefficient of variation over time (temporal variability: the ratio between the standard deviation of a variable and its mean value across replicates; Pimm 1984) of ecosystem respiration per microcosm. We then carried out a variance partitioning (i.e., assayed R^2 -value-based unique variance partitions for each predictor, i.e., assessed Type III Sums of Squares) for the two predictors, per experiment. In these simple two-way ANOVAs models, we did not correct for productivity because of the low statistical power of the tests. Depending on environmental conditions, AM

fungi can have strong or weak effects on plant biomass and ecosystem functioning (Johnson et al. 1997). The specific test aimed at assessing relative contributions (i.e., proportion of variability explained) to stability irrespective of the direction of the changes which the experimental parameter induced and allowed us to compare heterogenous parameters such as mycorrhiza with perturbations. To put our findings in perspective in relation to process rates, we additionally addressed changes in mean ecosystem respiration rates (i.e., we purposely did not use a repeated measures design so that we could later include experiment ID as a random effects factor) and had mean respiration rates per microcosm as a response variable in the series of ANOVAs we describe earlier.

To address hypothesis 2, we calculated the coefficients of variation over time for our experimental units. We worked with the subset of microcosms that had been exposed to no perturbators (i.e., no salinity stress, fertilization pulse, or defoliation disturbance). We fitted a general linear model with the following predictors: plant biomass (continuous predictor, log-transformed which was included in the model to relax concerns that it was biomass differences that were driving the results) and AM state (categorical predictor) and used the experimental ID as a categorical random effects factor. By default, R (R Core Team 2014) uses Type I sum of squares and the order in which predictors are fitted is important. For example, by fitting a predictor last, one obtains a lower sums of squares (and thus P values) for it than when fitted first. To approach our hypothesis in a conservative way, we fitted AM state of the microcosms in our analyses last and corrected for differences in plant biomass across microcosms as well as for differences in the mean CV across experiments.

To address hypothesis 3, we worked on the five experiments where we combined the manipulation of arbuscular mycorrhiza with that of one perturbator (i.e., NaCl, urea, or defoliation, representing manipulations of stress, pulse, and disturbance, respectively; experiments with IDs 5, 7, 8, 9, and 10 in Table 1). To assess resilience, we combined a metric of normalized system state with a metric of recovery time as suggested in Ingrisch and Bahn (2018). To calculate the normalized system state of resilience, we used the

approach of MacGillivray and Grime (1995) as follows:

$$U_{\text{state}} = \frac{P_x}{C_x} \quad (1)$$

where C_x and P_x stand for the ecosystem respiration rates in the controls and the treatment, respectively, at harvest x . The resilience is higher for larger values of U_{state} . An advantage of the specific metric of normalizing system rates is that it can integrate cases where the controls have lower ecosystem rates than the perturbed samples (e.g., after fertilization). We did four independent analyses, one for each of our harvests/assays. To further calculate a metric of recovery/trajectory time, we assessed time till observing a minimum in respiration. We did not assess time till full recovery because for some of the microcosms the system had not fully recovered by the end of our ecosystem respiration measurements, and for this reason, the specific analysis was only meant to be supplementary to that on normalized system rates. A long lag was evidence of a slow recovery of the system and indicated a low resilience. We normalized respiration rates with the three-replicate mean of the controls that had not received the perturbator and were of identical AM treatment. We used a paired t test (i.e., experiment was the pairing factor) to compare lags in AM and non-mycorrhizal (NM) microcosms. The specific metric of recovery time was limited by the fact that it assumed that recovery trajectories were similar across perturbations and was only used complementary to a commonly used in the literature method by MacGillivray and Grime (1995; formula 1).

RESULTS

Arbuscular mycorrhizal structures (AM fungal hyphae, vesicles, arbuscules) were only detected in plant roots in the +AM microcosms. The biomass in *P. lanceolata* microcosms varied between 12.4 and 1539 mg (quartiles 298–704) and in *P. vulgaris* between 3.4 and 136 mg (quartiles 13.5–136). *P. vulgaris* started growing approximately two weeks later than *P. lanceolata*, and while *P. lanceolata* plants without arbuscular mycorrhiza were 3.3% smaller than AM plants, *P. vulgaris* individuals became 45% larger after excluding AM fungi. The mean ecosystem

respiration rate in microcosms without plants was $2.32 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ compared to $3.05 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in microcosms with *P. vulgaris* and $6.18 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in microcosms with *P. lanceolata*. Respiration trajectories in the unplanted controls indicated the successful establishment of the microbial community before initiating the perturbations.

Microcosm plant biomass strongly influenced mean respiration ($F = 36.9$; $P < 0.001$; Appendix S2: Fig. S1c and Dp2), suggesting that we had to include plant biomass as a correction in our models of temporal variability. Arbuscular mycorrhiza ranked fourth in relative importance when predicting mean respiration with only a median 8% of variance explained by AM compared to 16% for the pulse (i.e., urea addition; Appendix S2: Fig. S1a). In the subset of planted microcosms that had received none of the three perturbations, even after correcting for plant biomass and plant species effects ($F_{1,43} = 10.544$; $P = 0.002$; Appendix S2: Dp2), we observed decreases in ecosystem respiration in the microcosms that were not

inoculated with AM fungi ($F_{1,43} = 10.5$; $P = 0.002$; Appendix S2: Fig. S1b). The relative importance of arbuscular mycorrhiza was higher than that of the three perturbations with regard to their contributions to temporal variability in the microcosms (the medians were 19% for AM, 15% for the pulse, 3% for the stress, and below 1% for the disturbance; Fig. 2). Again, there was a positive relationship between temporal variability and biomass ($F_{1,43} = 9.9$; $P = 0.003$, Fig. 3 insert; Appendix S2: Dp3) and excluding arbuscular mycorrhiza reduced by 37% temporal variability even after we corrected for the effects of biomass and plant species ($F_{1,43} = 8.51$; $P = 0.006$; Fig. 3; Appendix S2: Dp3).

In the third harvest, we observed an 83% decline in the metric of normalized system state (i.e., ecosystem respiration) following the exclusion of arbuscular mycorrhiza ($F_{1,24} = 15.8$; $P < 0.001$; Appendix S2: DP4; Fig. 4a, b). There were no differences in the normalized system state in the other three harvests (Appendix S2:

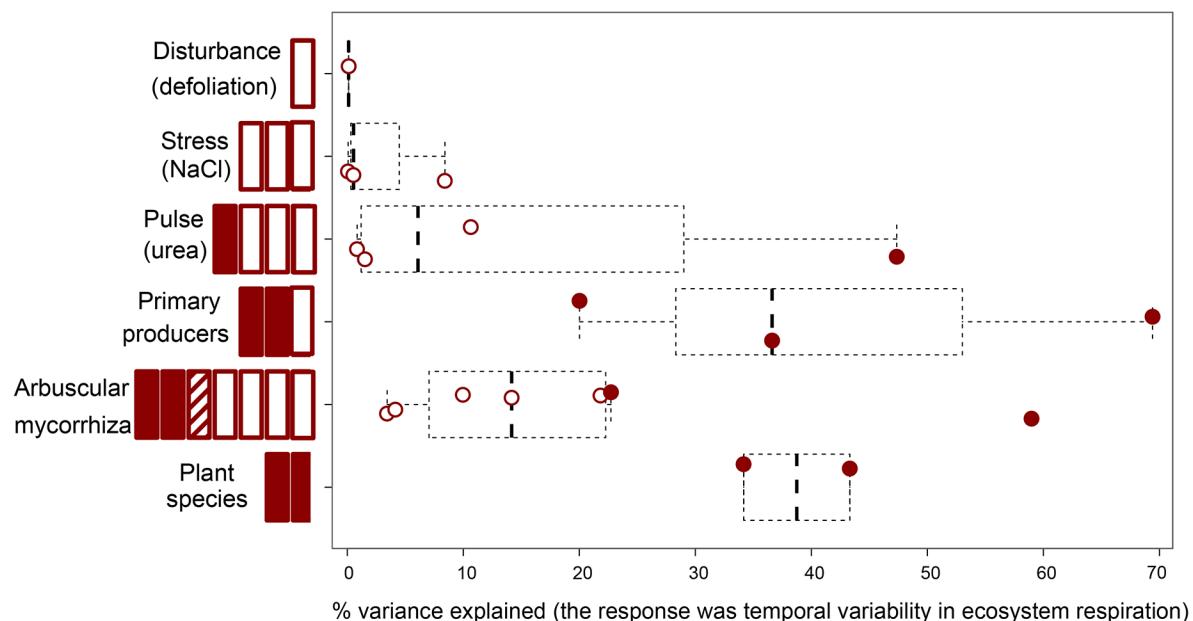


Fig. 2. Variance fractions explained by the six, that is, (1) disturbance through defoliating; (2) stress through adding NaCl; (3) pulse through fertilizing with urea; (4) presence/absence of plants which we describe as primary producers; (5) exclusion of arbuscular mycorrhiza; and (6) use of two different plant species *Plantago lanceolata* or *Prunella vulgaris*) experimental factors in the 10 bifactorial ANOVAs. The response variable in all cases was temporal variability, and the variance fractions were calculated as additive R^2 values (in all cases, $n = 12$). Rectangle/circle color depicts the significance of the predictors in the respective two-factor analysis of variance (filled rectangles, $P < 0.05$, striped rectangles, $0.05 < P < 0.1$, white rectangles, $P > 0.1$).

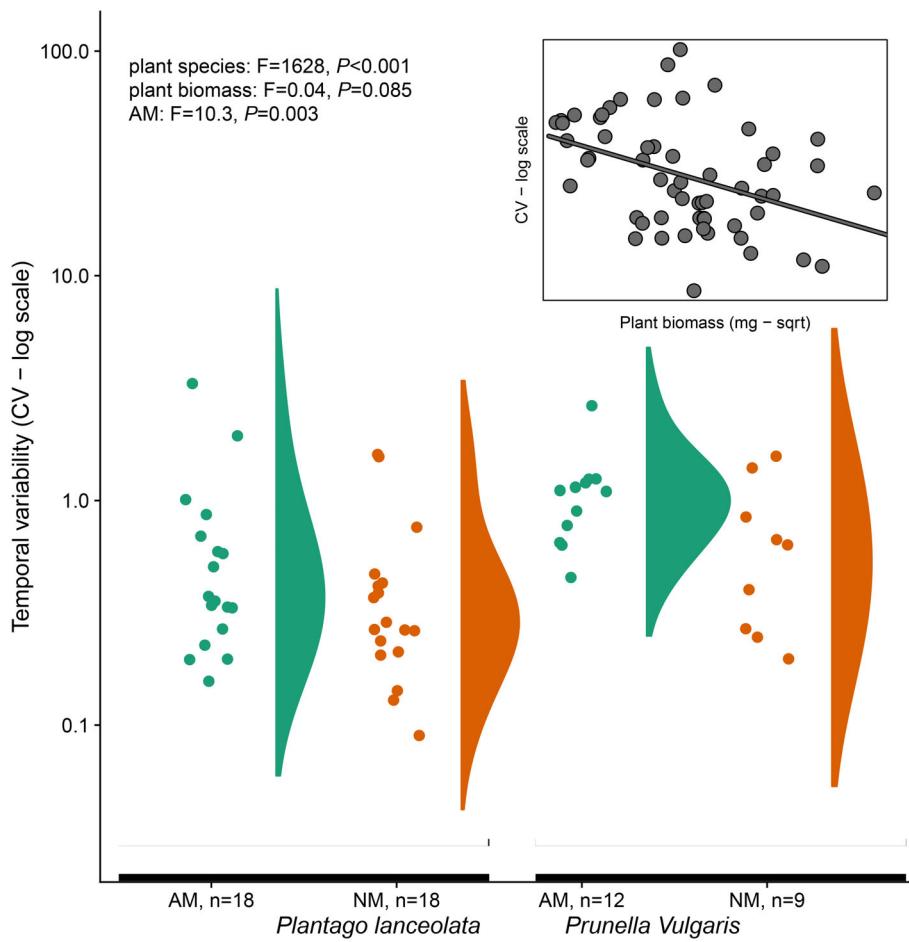


Fig. 3. Bee hive swarm plots depicting how temporal variability in microcosms that had not experienced perturbations varied with plant species (*Plantago lanceolata* or *Prunella vulgaris*) and arbuscular mycorrhizal state (AM, with arbuscular mycorrhiza; NM, without arbuscular mycorrhiza). The associated statistics of the model which includes corrections for plant biomass (i.e., insert) are presented on the top left of the figure. Low CV values implicate higher temporal stability.

DP4; because the time following a perturbation changes the resolution with which resilience is assayed, it was sufficient to detect differences in the normalized rate in one of the harvests). As an additional proxy of recovery time, we assessed the time it took to observe the minimum rates of ecosystem respiration after the manipulation (Fig. 4a–c) and it was in all cases quicker for AM. This correlated reasonably with actual resilience (i.e., full recovery of the system; Appendix S2: Fig. S3; we did not use actual resilience data because of the many missing values). We then carried out a paired t test to compare these

values which revealed a significant effect of AM ($t = -6.5; P = 0.003$; Appendix S2: DP5; Fig. 4c).

DISCUSSION

We present the first report in the literature addressing arbuscular mycorrhiza that disentangles between the two different forms of temporal stability, temporal variability and resilience. The combined analysis of 10 bifactorial experiments showed that the elimination of AM propagules in synthetic ecosystems reduces the temporal variability of ecosystem respiration, which was

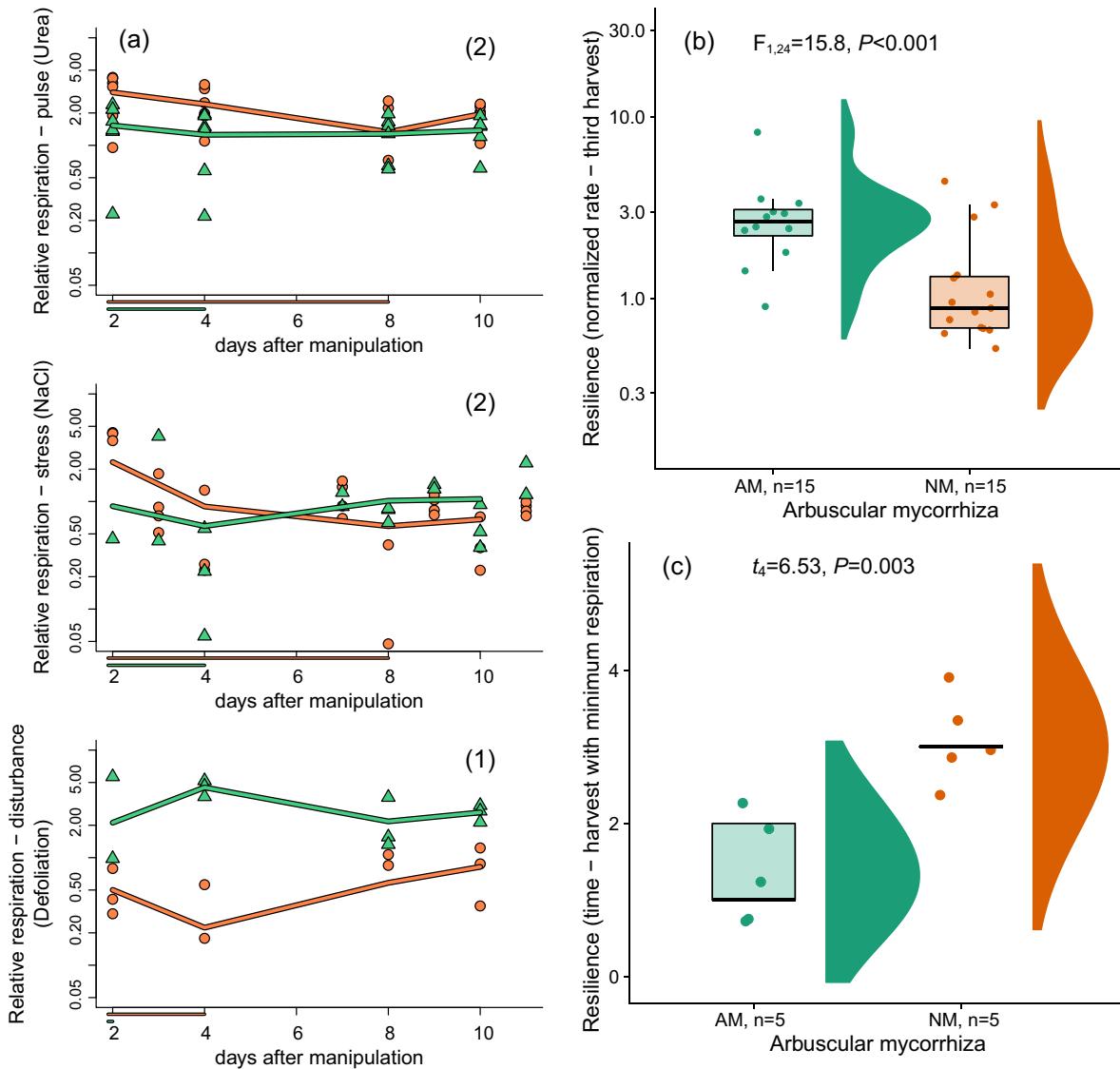


Fig. 4. Influence of arbuscular mycorrhiza on resilience: (a) Time (*x*-axis)—relative respiration (*y*-axis) diagrams for the subset of five controlled experiments where a mycorrhizal treatment was combined with a pulse, stress, or disturbance. We used these graphs to infer resilience of ecosystem respiration; here the harvest where we observed the minimum mean relative respiration value (which was a reasonable proxy of actual resilience; Appendix S2: Fig. S2). To assess relative respiration values, we standardized measurements from the microcosms that received the manipulations with the mean across all harvests of those that had not been treated and had the same mycorrhizal state. Green triangles stand for AM-inoculated microcosms whereas yellow cycles for NM microcosms. The overlaid continuous lines average across all observations per harvest (even if the harvests were asynchronous, i.e., panel b). Numbers in parentheses describe the number of experiments that are averaged. Note that the *y*-axis is plotted on a logarithmic scale whereas average values were calculated on a non-logarithmic scale. Colored bars behind the panels express time till the minimum observed respiration rate per AM or NM treatment. (b) Bee hive swarm plots on how a metric of resilience (normalized system state) differed in the microcosms with and without arbuscular mycorrhiza (c) bee hive swarm plots showing the distribution of time to minimal respiration across the abovementioned experiments. The difference between AM and NM microcosms was significant (paired *t* test, $t = -6.5$, $P = 0.003$).

relatively small in magnitude in our systems (Fig. 3). The resilience of ecosystem respiration to experimental perturbations, however, declined in the absence of arbuscular mycorrhiza suggesting that AM induces contrasting responses to temporal variability and resilience of the system.

Most of the existing work on temporal stability focuses on aboveground ecosystem responses and in particular plant biomass (de Mazancourt et al. 2013, Donohue et al. 2016, Caruso et al. 2018, Yang et al. 2018; but this gradually changes, e.g., Allison and Martiny 2008, de Vries et al. 2012, de Vries and Shade 2013, Griffiths and Philippot 2013). Such studies usually address the relationship between ecosystem stability and plant diversity (Tilman and Downing 1994, de Mazancourt et al. 2013, Loreau and de Mazancourt 2013, Gross et al. 2014). We here, alternatively, focused on a belowground process that can be measured non-destructively, ecosystem respiration. A key finding was that the relative importance of arbuscular mycorrhiza exceeded that of realistic levels of a pulse treatment (i.e., urea fertilization). This result was in agreement with hypothesis 1 stating that arbuscular mycorrhiza might be of lower importance for the temporal stability for ecosystem respiration than other experimental perturbations such as fertilization events. This might reflect (and be specific to) the experimental procedures and temporal scale of our experiments; nevertheless, this study represents the very first attempt to scale the impact of AM on temporal stability against other parameters. Most importantly, we also observed AM-induced changes in temporal variability in *P. vulgaris* despite its AM-growth depression (Fig. 3). Existing literature on how fertilization pulses alter temporal stability suggests strong effects but of an unknown direction. Wasson et al. (2017) showed that eutrophication lowered the resilience in a salt marsh whereas Yang et al. (2011) and Grman et al. (2010) found positive effects of fertilization on the temporal stability of terrestrial systems. By contrast, we found strong effects of net plant biomass and removal of plants in agreement with a study by Bruelheide and Luginbühl (2009). Exclusion of arbuscular mycorrhiza reduced temporal variability (Fig. 3), contrary to our expectations (hypothesis 2) and unlike the results of Yang et al. (2014). In our ranking of the relative importance of the factors,

we used in our experiments (Fig. 2) arbuscular mycorrhiza scaled below common manipulations such as plant species identity and inclusion of plants suggesting that it was of a relatively low importance to temporal stability (Fig. 2).

By contrast, manipulating arbuscular mycorrhiza drastically altered the resilience of the microcosms to our experimental perturbations (i.e., the three perturbators). This was apparent both in our normalized system state metric and our metric of recovery time, and these changes were in agreement with hypothesis 3. We think that this was because AM fungi promote plant host growth more effectively under suboptimal growth conditions (Johnson et al. 1997) which could cascade back to the ecosystem. Adding NaCl or defoliating the plants should have induced physiological changes to the plants, most likely exceeding those happening to the AM hyphal network (Miller et al. 1995), which reduced photosynthesis (Sultana et al. 1999) and rhizodeposition. It is likely that AM-inoculated plants could tolerate the specific levels of salinity (Augé et al. 2014) or defoliation better than uninoculated plants resulting in a faster recovery of the system. Additions of urea, via lowering rhizodeposition (Yoneyama et al. 2013), might have also been detrimental for ecosystem respiration in the rhizosphere, in which case improved scavenging of AM-inoculated plants for rhizodeposits might have sped up recovery from the perturbation.

Expectations on how excluding arbuscular mycorrhiza alters temporal stability might be conflicting (Fig. 1a). We observed contrasting changes in temporal variability and resilience which might help us reconcile our original predictions (Fig. 1b) with our observations. We proposed two reasons why excluding AM association reduces temporal variability: Symbiotic systems such as plant hosts colonized with Glomeromycotina have a lower structural stability (Allesina and Tang 2012) implying that they vary in relation to ecosystem process rates in time more than they would without their symbionts (i.e., when arbuscular mycorrhiza are excluded). Moreover, ecosystems without arbuscular mycorrhiza have been shown to lack specific equalizing mechanisms induced from AM associations but maintain additional stabilizing mechanisms compared to systems with arbuscular mycorrhiza (Veresoglou et al. 2018a).

Stabilizing mechanisms magnify niche differences (Carroll et al. 2011) and through promoting ecosystem functioning might favor temporal stability (i.e., portfolio effect: Yachi and Loreau 1999). By contrast, equalizing mechanisms lower relative fitness differences across organisms (Carroll et al. 2011) and can be detrimental for temporal stability because they intensify competition as a result (Douda et al. 2018). The net effect of excluding AM to these two types of coexistence mechanisms should be a higher temporal stability in the absence of arbuscular mycorrhiza (Fig. 1b). Following perturbations, however, excluding arbuscular mycorrhiza compromises the ability of the ecosystem to cope with stresses and disturbances (Smith and Read 2008). Moreover, because a plant without its AM symbionts makes a smaller holobiont (i.e., the assembly of different species into an ecological unit), it might cope less well with a perturbation of a given intensity (Lawton 1995; Fig. 1b). Despite a lower temporal variability in the absence of AM associations, excluding Glomeromycotina lowers resilience which is congruent with our observations.

We initially presented three hypotheses on the way arbuscular mycorrhiza could be influencing temporal ecosystem stability. We found evidence supporting two of the hypotheses (hypothesis 1 and 3, predicting that resilience declines after excluding AM fungi) but also a contrast in the way AM impacts temporal variability and resilience. This reflects how limited our current understanding of temporal ecosystem stability is, and in particular the role of a key mutualist, arbuscular mycorrhiza. By further studying the implications of structural constituents of ecosystems on their ability to absorb change, we may improve our predictive ability but possibly also our ability to mitigate the consequences of climate change.

ACKNOWLEDGMENTS

The study was funded by the Deutsche Forschungsgemeinschaft project Metacorrhiza (VE 736/2-1). We would like to thank the students of the course Lebensgemeinschaften und Biodiversität, led by SDV, for acquiring the data and Jason Hoeksema for commenting on the manuscript. A full list of the student is presented in Appendix S3, while primary data are presented as part of Appendix S4. SDV conceived the

study. SDV carried out the experiment, analyzed the data, and wrote the manuscript. The data were collected by students of the course Lebensgemeinschaften und Biodiversität, led by SDV. AM, MMA, MMo, and MF offered technical support during the experiment and supervised the measurements during the class. All authors contributed comments and approved the manuscript.

LITERATURE CITED

- Allesina, S., and S. Tang. 2012. Stability criteria for complex ecosystems. *Nature* 483:205–208.
- Allison, S. D., and J. B. H. Martiny. 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences USA* 105:11512–11519.
- Augé, R. M., H. D. Toler, and A. M. Saxton. 2014. Arbuscular mycorrhizal symbiosis and osmotic adjustment in response to NaCl stress: a meta-analysis. *Frontiers in Plant Science* 5:562.
- Bruelheide, H., and U. Luginbühl. 2009. Peeking at ecosystem stability: making use of a natural disturbance experiment to analyze resistance and resilience. *Ecology* 90:1314–1325.
- Butterfield, B. J. 2009. Effects of facilitation on community stability and dynamics: synthesis and future directions. *Journal of Ecology* 97:1192–1201.
- Carroll, I. T., B. J. Cardinale, and R. M. Nisbet. 2011. Niche and fitness differences relate the maintenance of diversity to ecosystem function. *Ecology* 92:1157–1165.
- Caruso, T., E. Hammer, S. Hempel, J. Kohler, E. K. Morris, S. D. Veresoglou, N. Opitz, J. Wehner, and M. C. Rillig. 2018. Assessing soil ecosystem processes – biodiversity relationships in a nature reserve in Central Europe. *Plant and Soil* 424: 491–501.
- Chaudhary, V. B., et al. 2016. MycoDB, a global database of plant response to mycorrhizal fungi. *Scientific Data* 3:160028.
- de Mazancourt, C., et al. 2013. Predicting ecosystem stability from community composition and biodiversity. *Ecology Letters* 16:617–625.
- de Vries, F. T., M. E. Liiri, L. Bjørnlund, M. A. Bowker, S. Christensen, H. M. Setälä, and R. D. Bardgett. 2012. Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change* 2:276.
- de Vries, F. T., and A. Shade. 2013. Controls on soil microbial community stability under climate change. *Frontiers in Microbiology* 4:65.
- Delgado-Baquerizo, M., D. J. Eldridge, V. Ochoa, B. Gozalo, B. K. Singh, and F. T. Maestre. 2017. Soil microbial communities drive the resistance of

- ecosystem multifunctionality to global change in drylands across the globe. *Ecology Letters* 20:1295–1305.
- Denny, M. W., B. Helmuth, G. H. Leonard, C. D. G. Harley, L. J. H. Hurt, and E. K. Nelson. 2004. Quantifying scale in ecology: lessons from a wave-swept shore. *Ecological Monographs* 74:513–532.
- Donohue, I., et al. 2016. Navigating the complexity of ecological stability. *Ecology Letters* 19:1172–1185.
- Douda, J., J. Doudová, J. Hulík, A. Havrdová, and K. Boublík. 2018. Reduced competition enhances community temporal stability under conditions of increasing environmental stress. *Ecology* 99:2207–2216.
- Eisenhauer, N., A. Milcu, E. Allan, N. Nitschke, C. Scherber, V. Temperton, A. Weigelt, W. W. Weisser, and S. Scheu. 2011. Impact of above- and below-ground invertebrates on temporal and spatial stability of grassland of different diversity. *Journal of Ecology* 99:572–582.
- Griffiths, B. S., and L. Philippot. 2013. Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews* 37:112–129.
- Grman, E., J. A. Lau, D. R. Schoolmaster, and K. L. Gross. 2010. Mechanisms contributing to stability in ecosystem function depend on the environmental context. *Ecology Letters* 13:1400–1410.
- Gross, K., B. L. Cardinale, J. W. Fox, A. Gonzales, M. Loreau, H. W. Polley, P. B. Reich, and J. van Ruijven. 2014. Species richness and the temporal stability of biomass production: a new analysis of recent biodiversity experiments. *American Naturalist* 183:1–12.
- Guelzow, N., F. Muijsers, R. Ptacnic, and H. Hillebrand. 2017. Functional and structural stability are linked in phytoplankton metacommunities of different connectivity. *Ecography* 40:719–732.
- Halley, J. M. 1996. Ecology, evolution and 1f-noise. *Trend in Ecology and Evolution* 11:33–37.
- Hodgson, D., J. L. McDonald, and D. L. Hosken. 2015. What do you mean, ‘resilient’? *Trends in Ecology and Evolution* 30:503–506.
- Holling, C. S. 1973. Resilience and stability of ecological systems. *Annual Reviews in Ecology Evolution and Systematics* 4:1–23.
- Ingrisch, J., and M. Bahn. 2018. Towards a comparable quantification of resilience. *Trends in Ecology and Evolution* 33:251–259.
- Jansa, J., F. A. Smith, and S. E. Smith. 2008. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytologist* 177:779–789.
- Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* 135:575–585.
- Lawton, J. 1995. Population dynamic principles. Pages 147–163 in J. Lawton and R. May, editors. *Extinction rates*. Oxford University Press, Oxford, UK.
- Leifheit, E. F., S. D. Veresoglou, A. Lehmann, E. K. Morris, and M. C. Rillig. 2014. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation – a meta-analysis. *Plant and Soil* 374:523–537.
- Loreau, M., and C. de Mazancourt. 2013. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. *Ecology Letters* 16:106–115.
- MacGillivray, C. W., and J. P. Grime. 1995. Testing predictions of the resistance and resilience of vegetation subjected to extreme events. *Functional Ecology* 9:640–649.
- May, R. M. 1972. Will a large complex system be stable? *Nature* 238:413–414.
- Miller, R. M., D. R. Reinhardt, and J. D. Jastrow. 1995. External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* 103:17–23.
- O’Connor, N. E., and L. Donohue. 2013. Environmental context determines multi-trophic effects of consumer species loss. *Global Change Biology* 19: 431–440.
- Pachepsky, E., T. Taylor, and S. Jones. 2002. Mutualism promotes diversity and stability in a simple artificial ecosystem. *Artificial Life* 8:5–24.
- Pellkofer, S., M. G. A. van der Heijden, B. Schmid, and C. Wagg. 2016. Soil communities promote temporal stability and species asynchrony in experimental grassland communities. *PLOS ONE* 11:e0148015.
- Pimm, S. L. 1984. The complexity and stability of ecosystems. *Nature* 307:321–326.
- Pouzols, F. M., T. Toivonen, E. di Minin, A. S. Kukkala, P. Kullberg, J. Kuusterä, J. Lehtomäki, H. Tenkanen, P. H. Verburg, and A. Moilanen. 2014. Global protected area expansion is compromised by projected land-use and parochialism. *Nature* 516:383–386.
- R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rillig, M. C., M. Wagner, M. Salem, P. M. Antunes, C. George, H. G. Ramke, M. M. Titirici, and M. Antonietti. 2010. Material derived from hydrothermal carbonization: effects on plant growth and arbuscular mycorrhiza. *Applied Soil Ecology* 45: 238–242.
- Roger, F., S. Bertilsson, S. Langenheder, O. A. Osman, and L. Gamfeldt. 2016. Effects of multiple dimensions of bacterial diversity on functioning, stability and multifunctionality. *Ecology* 97:2716–2728.

- Siddiky, M. R. K., J. Kohler, M. Cosme, and M. C. Rillig. 2012. Soil biota effects on soil structure: interactions between arbuscular mycorrhizal fungal mycelium and collembola. *Soil Biology and Biochemistry* 50:33–39.
- Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis. Third edition. Academic Press, Cambridge, Massachusetts, USA.
- Smith, R. A., T. R. Levine, K. A. Lachlan, and T. A. Fediuk. 2002. The high cost of complexity in experimental design and data analysis type I and type II error rates in multiway ANOVA. *Human Communication Research* 28:515–530.
- Sultana, N., T. Ikeda, and R. Itoh. 1999. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Environmental and Experimental Botany* 42:211–220.
- Thébaud, E., and C. Fontaine. 2010. Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* 329:853–856.
- Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. *Nature* 367:363–365.
- Tolkkinen, M., H. Mykra, A. M. Markkola, and T. Muotka. 2015. Human disturbance increases functional but not structural variability of stream fungal communities. *Functional Ecology* 29:1569–1577.
- van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:72–75.
- Veresoglou, S. D. 2015. P hacking in biology: an open secret. *Proceedings of the National Academy of Sciences USA* 112:E5112–E5113.
- Veresoglou, S. D., M. C. Rillig, and D. Johnson. 2018a. Responsiveness of plants to mycorrhiza regulates coexistence. *Journal of Ecology* 106:1864–4875.
- Veresoglou, S. D., E. Verbruggen, O. Makarova, I. Mansour, R. Sen, and M. C. Rillig. 2019. Arbuscular mycorrhizal fungi alter the community structure of ammonia oxidizers at high fertility via competition for soil NH₄⁺. *Microbial Ecology* 78:147–158.
- Vierheiling, H., A. P. Coughlan, U. Wyss, and Y. Piché. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* 64:5004–5007.
- Wasson, K., et al. 2017. Eutrophication decreases salt marsh resilience through proliferation of algal mats. *Biological Conservation* 212:1–11.
- Wurzburger, N., E. N. J. Brookshire, M. L. McCormack, and R. A. Lankau. 2017. Mycorrhizal fungi as drivers and modulators of terrestrial ecosystem processes. *New Phytologist* 213:996–999.
- Yachi, S., and M. Loreau. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences USA* 96:1463–1468.
- Yang, G., N. Liu, W. Lu, S. Wang, H. Kan, Y. Zhang, L. Xu, and Y. Chen. 2014. The interaction between arbuscular mycorrhizal fungi and soil phosphorus availability influences plant community productivity and ecosystem stability. *Journal of Ecology* 102:1072–1082.
- Yang, Z. L., J. van Ruijven, and G. Z. Du. 2011. The effects of long-term fertilization on the temporal stability of alpine meadow communities. *Plant and Soil* 345:315–324.
- Yang, G., C. Wagg, S. D. Veresoglou, S. Hempel, and M. C. Rillig. 2018. How soil biota influence ecosystem stability. *Trends in Plant Science* 23:1057–1067.
- Yoneyama, K., X. Xie, T. Kisugi, T. Nomura, and K. Yoneyama. 2013. Nitrogen and phosphorus fertilization negatively affects strigolactone production and exudation in sorghum. *Planta* 238:885–894.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3308/full>